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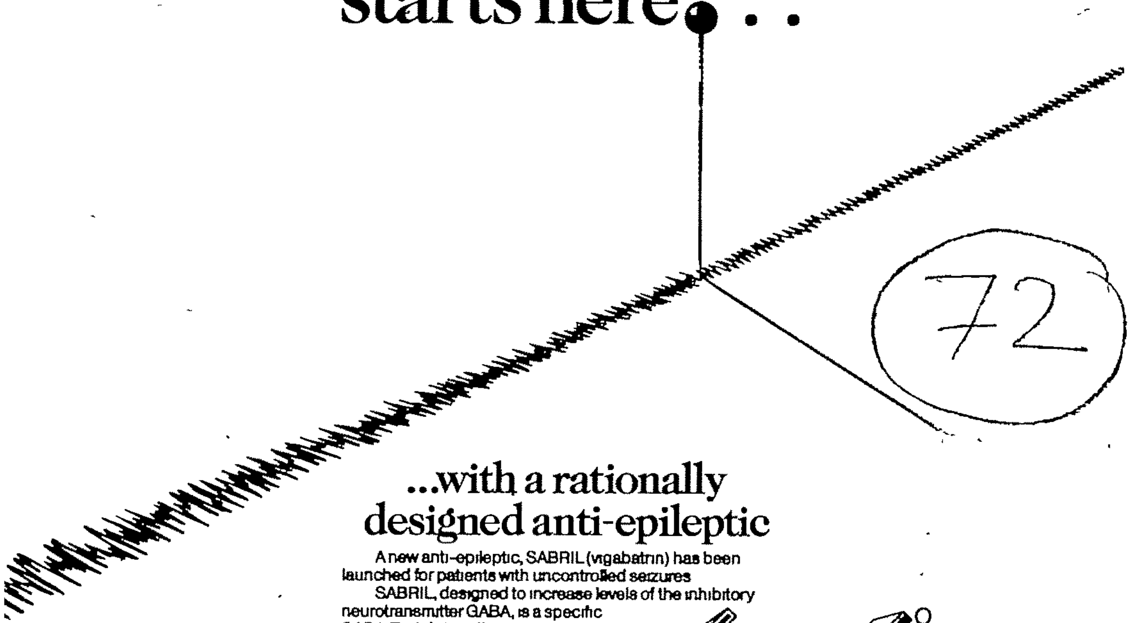

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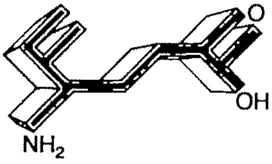

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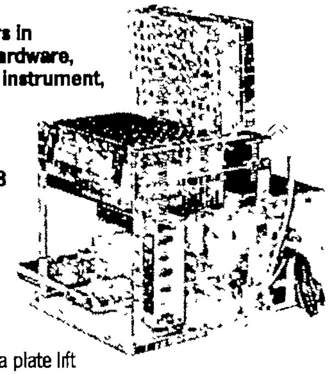

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ACUTE DISSEMINATED ENCEPHALOMYELITIS

MRI FINDINGS AND THE DISTINCTION FROM MULTIPLE SCLEROSIS

by JÜRG KESSELRING, DAVID H. MILLER, STEPHANIE A. ROBB,
BRIAN E. KENDALL, IVAN F. MOSELEY, DEREK KINGSLEY,
E. P. GEORGE H. DU BOULAY *and* W. IAN MCDONALD

(From the Multiple Sclerosis NMR Research Group, Institute of Neurology and the National Hospital, Queen Square, London)

SUMMARY

Brain MRI was performed on 12 patients with acute disseminated encephalomyelitis (ADEM). Multifocal white matter lesions indistinguishable from those seen in multiple sclerosis (MS) were found in 10. In 5 there were rather extensive symmetric abnormalities in the cerebral (2) or cerebellar white matter (2), or basal ganglia (1). Follow-up MRI after intervals of 2 wks to 18 months demonstrated partial resolution of the abnormalities, but some persisting lesions. New MRI lesions were found at follow-up in only 1 case after an interval of 2 wks Serial MRI makes a useful contribution to the distinction between MS and ADEM.

INTRODUCTION

Acute disseminated encephalomyelitis (ADEM) and multiple sclerosis (MS) are both inflammatory demyelinating diseases of the central nervous system (CNS). Whereas ADEM is usually a monophasic illness, MS is by definition a multiphasic disease which frequently results in stepwise or steadily progressive deterioration in neurological function. For this reason differentiation of ADEM from MS in a patient with a single clinical episode attributable to CNS demyelination is of prognostic importance.

Magnetic resonance imaging (MRI) demonstrates white matter lesions with a high sensitivity both in MS (Young *et al.*, 1981; Ormerod *et al.*, 1987; Paty *et al.*, 1988) and ADEM (Atlas *et al.*, 1986). This paper presents the MRI findings in a group of patients with ADEM, and discusses the role of the MRI in the distinction of ADEM and MS.

PATIENTS AND METHODS

The patients reported in this study were seen at the National Hospitals for Nervous Diseases, Maida Vale and Queen Square, or at the Hospital for Sick Children, Great Ormond Street, London, between 1985 and 1987. ADEM was diagnosed by the development of a CNS white matter syndrome occurring in close temporal relationship with a systemic illness which had clinical characteristics of viral or mycoplasma infection. Twelve cases were thus identified, in whom the systemic illness preceded the neurological syndrome in 11, while in 1 case (*see* Table, Case 1) typical mumps developed 8 days after

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TABLE CLINICAL DATA, INVESTIGATIONS AND FOLLOW-UP

Case	Sex	(yrs)	Infection	Clinical syndrome at presentation	CSF	Initial MRI	Follow-up		
							Interval (months)	Clinical	MRI
1 (A.A)	F	5	Mumps	Bilateral visual failure and flaccid tetraplegia	Normal	Multiple pv and dc	5	Complete recovery	Partial resolution of lesion particularly of pv. No new lesions
2 (B K)	M	19	Mycoplasma	Flaccid tetraplegia involvement	Cells ↑	nd	2	Unchanged	pv+dc Persistence of WM lesions; atrophy
3 (B L)	F	39	Chickenpox	Drowsiness, gait disturbance	Cells ↑, OB, free light chains	dc, no pv	18	Complete recovery	Partial resolution of lesions No new lesions
4 (D A.)	F	25	Mumps	Psychosis	nd	Multiple pv and dc	3/18	Complete recovery; 3 months of psychosis at 18 months	Persistence of initial lesions on both follow-ups No new lesions
5 (McG J)	M	7	Nonspecific upper respiratory tract infection	Truncal and limb ataxia. VI nerve palsy Left extensor plantar response	nd	Extensive cerebral and cerebellar hemisphere WM lesions	12	Almost complete recovery	Incomplete resolution of lesions No new lesions
6 (S C.)	F	4	Upper respiratory tract infection	Tetraparesis and ataxia	Cells ↑, protein ↑, OB	Multiple confluent pv, dc, Extensive cerebellar hemisphere lesions	4/12	Almost complete recovery	Marked but incomplete resolution of lesions. No new lesions
							2	Complete	Resolution of basal ganglia

7 (H.D.)	M	6	Sore throat	Bilateral optic neuritis Initially drowsy and irritable. Large central scotomas	nd	Swelling cervical cord. Symmetric changes in basal ganglia	2	recovery	and of some discrete cerebral WM lesions
8 (H D)	M	32	Adenovirus	Tetraparesis below T1 Vertical diplopia. Bilat. facial nerve palsy	Cells ↑, OB	Several dc, pv, parietal, middle cerebellar peduncle, pons	2/6	Marked improvement	Marked, but incomplete resolution. No new lesions
9 (R M.)	M	31	Upper respiratory tract infection	Flaccid paraparesis with retention of urine	Cells ↑, protein ↑	Extensive occipital WM changes L>R	12	Marked improvement	Incomplete resolution of WM changes
10 (C D)	M	14	Mycoplasma	Flaccid tetraplegia requiring respiratory support	Cells ↑, protein ↑	Single dc cerebral WM lesion, swelling and extensive high signal in cervical cord	1–2 weeks 3 months	Unchanged	2 weeks. reduction in size of initial cerebral lesion, appearance of several new dc and confluent WM lesions 3 months: marked atrophy of cervical cord. No new lesions
11 (H D)	M	6	Chickenpox	Bilateral visual failure	nd	Symmetric high signal areas in parietal-occipital WM (very extensive)	nd		
12 (T S)	F	21	Chickenpox	Upward gaze palsy. Nystagmus	nd	nd	1	Marked improvement	Isolated high signal lesion in midbrain

WM, = white matter; pv = periventricular lesions, dc = discrete cerebral lesions, nd = not done, OB = oligoclonal IgG bands.

the onset of the neurological symptoms. Detailed clinical reports of the 2 cases following mycoplasma infection have been reported elsewhere (Francis *et al.*, 1988); the 2 cases after chickenpox infection were mentioned briefly in another publication (Miller *et al.*, 1990).

Brain MRI was performed on all patients using a Picker 0.5T superconducting magnet. In all cases a moderately T_2-weighted sequence ($SE_{2000/60}$) was performed. Additional more heavily T_2-weighted ($SE_{2000/120}$) or T_1-weighted ($IR_{2000/40/500}$) images were usually obtained. The brain was imaged with 5 or 10 mm contiguous axial slices. Follow-up MRI studies were obtained in 10 cases after an interval of two wks to 18 months.

RESULTS

Clinical features (see Table)

Eight patients (Cases 1 – 8) had clinical evidence of a multifocal neurological syndrome compatible with ADEM. In the 4 other cases there was clinical evidence of only a single lesion: transverse myelitis (Cases 9 and 10), bilateral optic neuritis (Case 11), and a brainstem syndrome (Case 12).

CSF

The CSF was normal in Case 1 and showed elevated cells and protein in the remaining 6 patients in whom it was examined. In the only patient (Case 8) in whom lumbar puncture was performed repeatedly, oligoclonal IgG bands were present only on the third of five occasions.

MRI

Brain MRI was abnormal in all 12 cases in the acute stage of the illness. In 11, there were multifocal white matter lesions in the cerebral hemispheres. Asymmetric and multifocal periventricular and discrete white matter changes resembling those of MS were seen in 10 (fig. 1). In Case 12 there was a single lesion in the midbrain, but no supratentorial abnormality.

In 8 cases, there were some features of the distribution of the lesions which differed from that usually seen in MS. Five showed extensive symmetric bilateral changes: in 2 these changes were confined to the occipital or parieto-occipital white matter (fig. 2); in 2 they involved the cerebellar peduncles or cerebellar hemispheres (fig. 3A,B); and in 1 they involved the basal ganglia and hypothalamus (fig. 4). Three other cases displayed multiple discrete lesions in the cerebral white matter with only minor periventricular changes (fig. 5). Such a pattern is relatively unusual for MS (Ormerod *et al.*, 1987).

Follow-up MRI after 2 wks to 18 months in 10 cases showed either no change or more commonly a variable degree of resolution of the changes, although in all cases some lesions persisted. New lesions were seen in 1 case (Case 11). This was a 14-yr-old boy who was first scanned within 3 days of the onset of an acute spinal syndrome following mycoplasma infection. MRI revealed a subcortical lesion in the right frontal lobe. Two weeks later, this lesion was beginning to resolve, but several new periventricular lesions had appeared. The patient had continued to deteriorate during

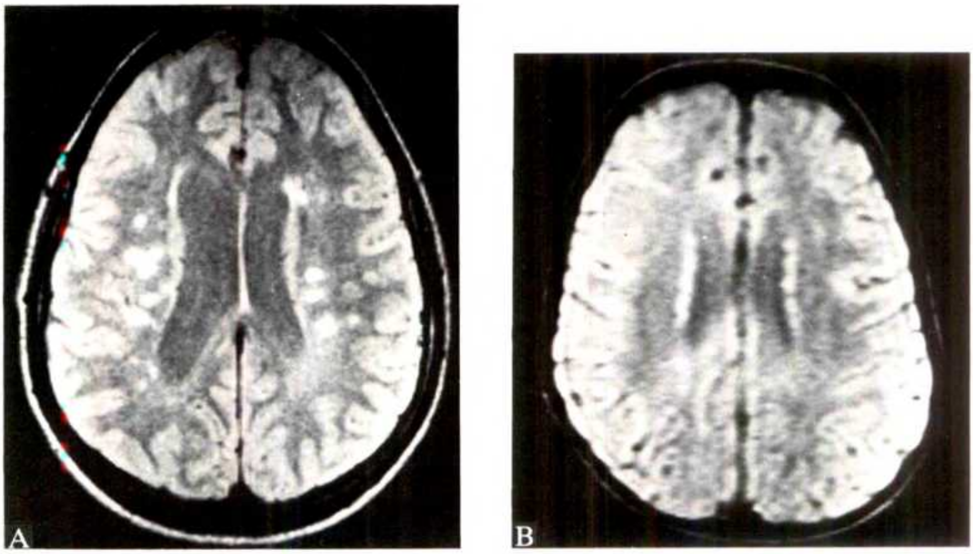

FIG. 1. MRI: spin echo sequence ($SE_{2000/60}$). A, multiple lesions in the cerebral white matter, several aligned along the edge of the ventricle (*Case 2*); B, less extensive periventricular changes (*Case 1*).

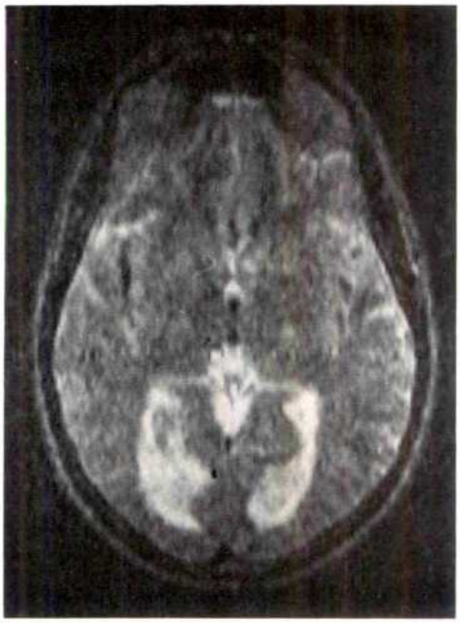

FIG. 2. MRI: moderately T_2-weighted spin echo sequences ($SE_{2000/80}$). Large symmetric areas of high signal intensity in both occipital lobes in a patient with acute clinically complete transverse myelitis after an unspecific upper respiratory tract infection (*Case 9*).

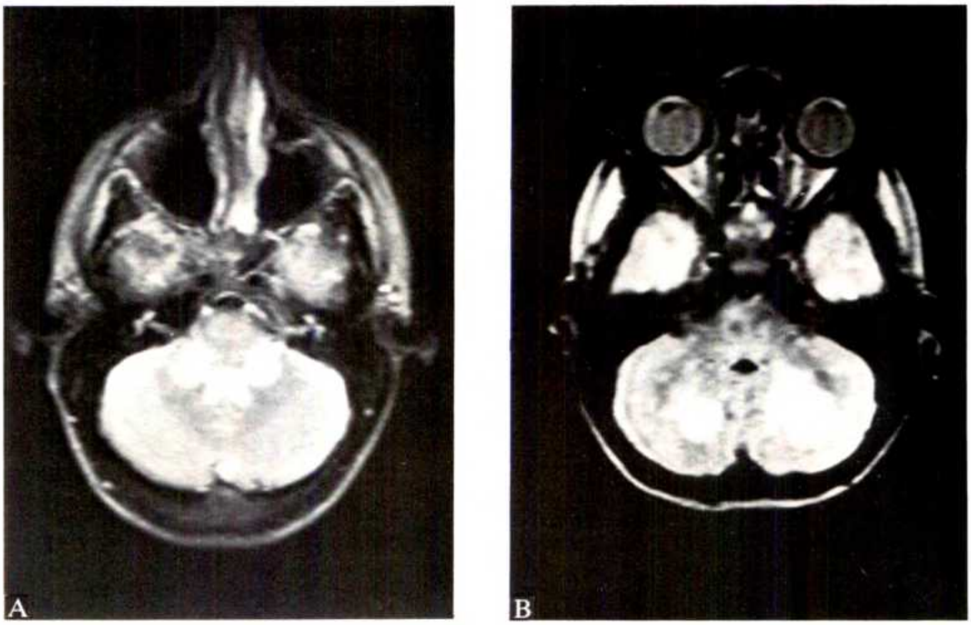

FIG. 3. MRI: T_2-weighted spin echo sequence ($SE_{2000/120}$). A, large symmetric areas of high signal intensity in the brachium pontis and the adjacent cerebellar white matter in a 32-yr-old man with postadenovirus tetraparesis (*Case 8*). B, large areas of high signal intensity in the cerebellar white matter on both sides in a 4-yr-old girl with tetraparesis and severe ataxia after an unspecific upper respiratory tract infection (*Case 6*).

this period. After 3 months MRI showed partial resolution of all the earlier lesions and no further new lesion had appeared.

Illustrative cases

Case 3

History. Two children of a 36-yr-old housewife had recently had chickenpox. Ten days before admission to hospital, she herself developed a pyrexia and the typical chickenpox rash. Over 3 days she developed generalized headache, nausea, photophobia and unsteadiness of gait.

Examination. She was drowsy but alert and orientated. There was a typical chickenpox rash. The fundi were normal. Eye movements showed some impairment of pursuit and the occurrence of square-wave jerks. On lying down there was occasional downbeat nystagmus. Power, tone and sensation were normal in all limbs, but there was marked ataxia of gait and of the limbs, especially on the left.

Investigations. Cranial CT and blood examinations were normal. EEG showed mild bilateral abnormalities. In the CSF there were 50 mononuclear cells/mm^3, and the protein content was slightly elevated (0.52 g/l). CSF electrophoresis showed oligoclonal immunoglobulins and elevated numbers of free light chains. MRI showed several discrete lesions in the cerebral white matter without any noticeable periventricular changes (fig. 5), and a small area of high signal in the medulla.

Outcome. Following treatment with dexamethasone, the patient made a full recovery within 14 days. On follow-up 18 months later she had remained free of symptoms, and neurological examination was normal.

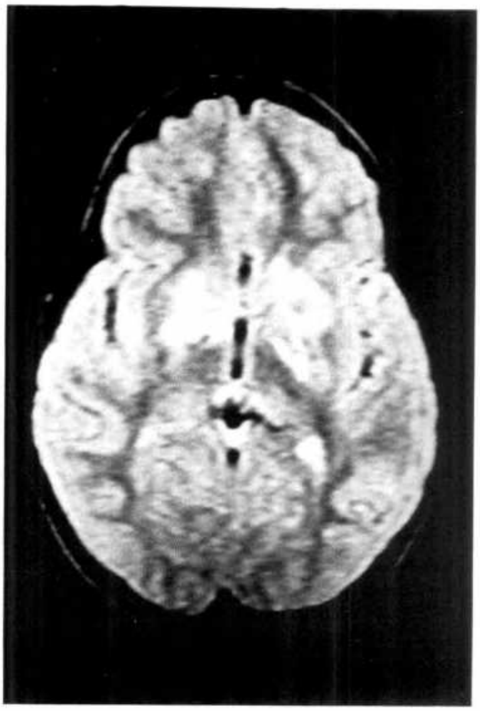

FIG. 4. MRI: T_2-weighted spin echo sequence ($SE_{2000/120}$). Areas of high signal intensity in the basal ganglia and hypothalamus in a 6-yr-old boy with acute bilateral visual failure (*Case 7*).

On MRI, some lesions which had been visible on the initial scan had partially resolved but several lesions persisted. No new lesion had appeared.

Case 7

History. A 31-yr-old technical assistant experienced a 3–4 day illness of a 'flu-like' nature with fever, aching joints and lymphadenopathy. He recovered completely but after 2 wks he developed pain in the right leg. Over the course of one day the right leg became numb and weak, and he was unable to walk. Retention of urine necessitated catheterization. He then experienced a constant pain in the thoracic region which was exacerbated by deep breathing.

Examination. There was complete flaccid paraplegia with absent tendon reflexes on the lower limbs; the plantar responses were unobtainable. There was retention of urine. A sensory level for all modalities initially at T10 rose over a few days to T4.

Investigations. A (traumatic) lumbar puncture showed 190 red and 86 white cells/mm^3, an elevated protein content (1.05 g/l), but no oligoclonal IgG bands. Somatosensory-evoked potentials were consistent with a lesion in the thoracic spinal cord. Cranial CT revealed extensive bilaterally symmetric low density areas in the deep white matter of both occipital lobes without contrast enhancement. MRI, 2 wks after the initial neurological symptoms, showed increased signal intensity symmetrically in the occipital periventricular white matter (fig. 2A).

Course. The patient failed to improve significantly and was discharged after 4 wks to a spinal injury centre. One year later he was examined and scanned again: there was marked improvement clinically and he was back at work. The initial MRI lesions had resolved to a marked degree, although several discrete cerebral white matter lesions had persisted.

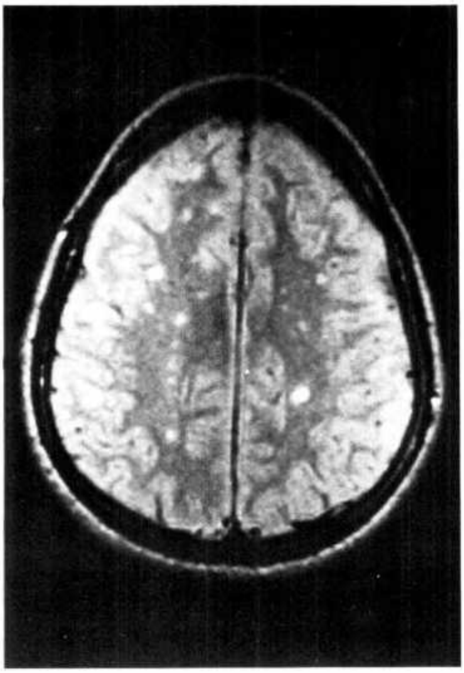

FIG. 5. Multiple discrete white matter changes without periventricular abnormalities in a 39-yr-old woman with drowsiness and gait disturbance after chickenpox infection (*Case 3*).

Case 11

History. Eleven days before admission to hospital, this 32-yr-old man developed a high temperature and a sore throat. After an initial recovery with ampicillin there was recurrence of fever 1 wk later associated with an aching pain in both groins and difficulty in passing urine. Over the course of a day, he developed complete flaccid paralysis of the lower limbs together with an ascending numbness from his toes to the waist. Over the next 2 days the sensory disturbance ascended upwards with numbness and incoordination in the arms. He developed dysphagia.

Examination. The patient was ill with some neck stiffness. There was first degree nystagmus to right and left, dysarthria, and weakness of neck flexion. There was moderate pyramidal weakness in the upper limbs and a complete flaccid paraplegia with absent knee and ankle jerks. There was sensory loss below C4.

Investigations. Serum antibody titres against adenovirus (1/256 and 1/512) were elevated. Cranial CT, myelography, and nerve conduction studies were all normal The EEG showed moderate widespread nonspecific abnormalities. Five lumbar punctures were performed over a period of 2½ weeks: the number of cells declined from an initial value of 337 to $3/\text{mm}^3$ and the protein content varied between 0.60 and 2.20 g/l. Oligoclonal IgG bands were detected on one occasion during the first week of the illness, but not thereafter. Initial MRI was abnormal with discrete lesions of high signal intensity in both parietal lobes, and in the posterior limbs of both internal capsules. In the posterior fossa, areas of abnormal signal intensity could be demonstrated symmetrically in the middle cerebellar peduncles on both sides (fig. 3A) and in the central pons.

Course. One week later the patient experienced a further deterioration with nausea and vertical diplopia, and tingling paraesthesiae in the arms. The nystagmus was more marked and there was now facial weakness. Treatment with corticosteroids was begun and the patient's condition steadily improved. Two months after admission there was a mild spastic paraparesis and truncal ataxia. There was subjective sensory impairment below T6 and urinary incontinence. After 6 months the patient had further improved clinically, and on MRI the lesions in the brain seen previously had become less prominent but many were still visible. A few had disappeared entirely.

DISCUSSION

The distinction between ADEM and MS is of prognostic significance and is often not too difficult. Problems may arise, however, when there is a polysymptomatic presentation in the absence of a preceding infection, and in patients presenting with an isolated clinical deficit of a kind seen in both diseases, such as optic neuritis.

Certain clinical features may help to differentiate the two conditions. ADEM often produces a widespread CNS disturbance with coma or drowsiness, seizures, and multifocal neurological signs implicating the brain, spinal cord and optic nerves. In contrast, MS usually presents as a monosymptomatic syndrome such as optic neuritis or a subacute myelopathy. ADEM may also present in this way (McCarthy and Amer, 1978; Selbst *et al.*, 1983), although with some differences. Thus optic neuritis in ADEM is usually simultaneously bilateral (Miller *et al.*, 1986) whereas in MS it is more often unilateral; myelopathy in MS is frequently partial (Miller and Evans, 1953), but in ADEM it is often complete and associated with areflexia. Nevertheless no clinical feature is exclusive to one or other disorder. Thus a more diffuse 'encephalopathic' disturbance may rarely be seen with MS (Matthews, 1985), as may bilateral simultaneous optic neuritis (Parkin *et al.*, 1984) or a severe transverse myelitis. Furthermore, while ADEM is typically preceded by an infection (most often viral) or vaccination (Johnson *et al.*, 1985), relapse in MS may also follow such events (Sibley *et al.*, 1985).

Changes in the CSF also do not permit reliable differentiation of the two conditions. While a moderate CSF pleocytosis is usual in ADEM, high cell counts may occasionally be seen in MS; and although oligoclonal IgG bands are a characteristic feature of MS, they may also occur in ADEM, as they did in 3 cases in the present study. Since in MS, however, oligoclonal bands almost always persist, their disappearance (as in our patient who had serial lumbar punctures) is a useful distinguishing feature.

Thus it is not possible in a single clinical episode to make a certain distinction between MS and ADEM either on clinical features or CSF examination. It is therefore of interest to assess the diagnostic value of MRI in this context. The present study revealed an MRI pattern of multifocal asymmetric white matter lesions in the brain, indistinguishable from that seen in MS, in the majority of patients with ADEM. A similar result has been reported in two recent MRI studies of a total of 8 other cases of ADEM (Atlas *et al.*, 1986; Dunn *et al.*, 1986). It is not currently possible to determine the age of white matter lesions on a single scan. It follows, therefore, that the pattern demonstrated on MRI does not allow a distinction between MS and ADEM in patients with a single episode of neurological disturbance.

Recent MRI studies have revealed multifocal asymmetric white matter lesions indistinguishable from MS in 50–70% of adults presenting with clinically isolated syndromes involving the optic nerve (Jacobs *et al.*, 1986; Ormerod *et al.*, 1986*a*), brainstem (Ormerod *et al.*, 1986*b*) and spinal cord (Miller *et al.*, 1987). MS frequently presents as one or another of these three syndromes. In white adults living in temperate zone, where MS is common and ADEM rare, it is likely that MS is the cause of the MRI abnormalities in most cases. However, it is not possible at presentation to exclude the alternate diagnosis of ADEM in any individual case. The distinction of MS from ADEM on a single scan in these patients might be facilitated if there were reliable means of determining the age of lesions. In MS, lesions would be of a varying age; in most patients with ADEM, they would all be the same age. Studies in MS using gadolinium-DTPA enhanced MRI show a mixture of enhancing and nonenhancing lesions (Grossman *et al.*, 1986; Miller *et al.*, 1988), and there is evidence that enhancement (which indicates an abnormal blood-brain barrier) is a consistent feature of new and active lesions (Miller *et al.*, 1988). Given that ADEM is usually a monophasic disease, it might be expected that all lesions would enhance in the acute phase, while none would do so in the chronic phase. Gadolinium-DTPA enhanced MRI may therefore prove useful in distinguishing monophasic and multiphasic disease, but a study of enhancement in ADEM has yet to be performed.

Our study demonstrates certain patterns of MRI abnormality in ADEM which would be unusual in MS. In particular, there was a pattern in some cases of extensive and relatively symmetric abnormalities in the cerebral and cerebellar white matter, and in one case in the basal ganglia, a rare finding in MS (Ormerod *et al.*, 1987); cerebellar abnormalities were also noticed in one case by Dunn *et al.* (1986). These changes accord with the pathological findings in ADEM of diffuse symmetric perivenous inflammation and demyelination involving especially the cerebral white matter. In comparison, the pathological findings in MS are more typically those of asymmetric and multifocal demyelinating lesions of variable size (Allen, 1984).

Although there was considerable resolution of changes at follow-up, it was notable that some MRI abnormalities persisted for as long as 18 months, despite full clinical recovery. Persistent visualization of lesions is perhaps not surprising, given the pathological evidence for astrocytic hyperplasia and gliosis at a late stage of ADEM (van Bogaert, 1950; Allen, 1984). This finding also raises the possibility that some of the asymptomatic white matter lesions seen on MRI in healthy individuals may be due to past illness such as childhood exanthemata.

Serial MRI offers help in differentiating monophasic from multiphasic disease. New lesions are commonly seen after scanning intervals as short as a month (Miller *et al.*, 1988; Paty *et al.*, 1988; Willloughby *et al.*, 1989). New lesions would not be expected in ADEM, provided there is a sufficiently long follow-up interval. However, ADEM occasionally evolves over several weeks (new MRI lesions were seen in one of our patients after 2 wks) and rarely episodes of central neurological disturbance recur for up to 18 months after a typical attack of ADEM in children or adults without subsequent development of MS after many years of follow-up (Miller and Evans, 1953; Alcock and Hoffman, 1962). The interval after which it may be concluded with safety that the failure of new lesions to appear indicates that the patient has ADEM and not MS is at present uncertain. The criteria of the Poser committee (Poser *et al.*, 1983) require a minimum of 1 month for separate episodes to qualify as relapses of MS. We believe that it should be rather longer; our present practice is to allow a minimum of 6 months when the clinical illness and the CSF changes are more typical of ADEM. The experience of Miller and Evans (1953) suggests that in patients with unusual features a decision about the diagnosis should be delayed for as long as 2 yrs.

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THE CORTICOSPINAL TRACTS IN MAN

COURSE AND LOCATION OF FIBRES AT DIFFERENT SEGMENTAL LEVELS

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SUMMARY

The course, location and relations of the corticospinal tracts within the spinal cord of man are demonstrated on the basis of cases with lesions above the spinal cord restricted to the corticospinal tracts, of motor neuron disease, and of anterolateral cordotomies; control cases were of normal spinal cords.

The following features of the lateral corticospinal tract are emphasized in the cervical cord: (1) the large extent of the white matter of the cord covered by the tract, and the anterior extent of the tract, the border being anterior to the central canal; (2) in the lower cervical cord, the separation of fibres from the main mass of the tract, which reach the periphery of the cord in the anterolateral sector; (3) the presence in many cords of the ventral crossed bundle; and (4) the relationship of the denticulate ligament to the tracts in the cervical segments. The following features of the anterior corticospinal tracts are emphasized: (1) their location, caudal extent and asymmetry; and (2) the changes in location in relation to the median fissure as the tract descends and its relationship to other tracts of the anterior column. Three-quarters of spinal cords are asymmetric and in three-quarters of asymmetric cords the right side is the larger. The asymmetry is due to a greater number of corticospinal fibres crossing to the right side. As more fibres have crossed in the decussation, the anterior tract opposite the large lateral tract is smaller than the ipsilateral anterior tract: that accounts for the asymmetry of the two halves of the cord. The greater number of corticospinal fibres in the right side of the cord is unrelated to handedness, but correlates with the fact that in three-quarters of corticospinal decussations, the crossing from left to right occurs at a more cranial level than the opposite crossing.

A group of short peripheral ascending fibres is described running along the sides of the median fissure in the thoracic cord.

INTRODUCTION

The work presented here reports the course and relations of the corticospinal tracts in the human spinal cord. Although we and all other neurologists assumed that these were completely known, when studying the extent and location of descending tracts from the brainstem and propriospinal fibres, we found groups of fibres in the cervical cord lying anterior to the location classically attributed to the lateral corticospinal tract that were corticospinal. We also observed other features of the anterior tracts and the frequency of asymmetry of the cord due to asymmetry of the corticospinal tracts that were unknown or insufficiently known and that needed to be established, both from the anatomical and clinical standpoints.

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The older literature on the corticospinal tracts was largely covered by our review of 1955 (Nathan and Smith, 1955) and so it will not be mentioned here, nor will we discuss the larger literature on species other than man. For the corticospinal tracts, it is surprising that the facts concerning their course, topographic features, and area occupied on transverse sections presented in anatomical textbooks are meagre. Usually a diagram is given showing a general view of the tracts and it is implied that the location and relationships remain the same throughout the spinal cord. The lateral corticospinal tract is usually shown restricted to the posterior part of the lateral funiculus, and yet it has often been observed since the beginning of the present century that the tract comes well into the anterior part of the funiculus in the cervical cord. The extent of the tract is shown differently in various textbooks. The anterior tract is shown either along the border of the median fissure deep in the white matter of the anterior column or up against the medial border of the anterior horn. Although variations at the pyramidal decussation are well known, insufficient attention is paid to asymmetries between the two sides and their clinical implications.

In fact the topography of the spinal cord, except for the T2 – T12 segments, is different in every segment and even within one segment. These features were illustrated by Bruce (1901) in his Atlas. Our own observations, illustrated below, agree completely with his. The topography is so characteristic for each cervical, upper thoracic and lumbosacral segment of the cord that the precise level of any section can be deduced.

As this is a study of the corticospinal tract of man, the histological techniques used are necessarily those that can be applied to man. The new and rewarding techniques of studying the anatomy of the nervous system by using axonal transport in the living neuron cannot be applied to man.

In the subject of anatomy, the course of fibres or a tract is not very important: what is needed to be known is which group of neurons is connected to which other groups. But for clinical neurology, the course and the location of a tract is important. For, although lesions may damage cells of origin or the terminations of tracts, they are more likely to damage fibres during their course. Knowledge of the course, location and extent of a tract is needed in neurology and even more in neurosurgery. The course of tracts is not shown by the new methods but it is by the traditional degeneration techniques. One of the inherent disadvantages is that it is necessary to wait for appropriate lesions and for the lesions to occur at the right time for the histological techniques that can be used. The extent of the area occupied by a tract—and this is one of the features to which we will be paying attention—is found to differ slightly according to the period of survival and the staining method used. The area is more correctly assessed during the stage of degeneration. For by the time total loss of fibres occurs, the area appears to be smaller; in some cases a tract consisting of only a few fibres can be identified only when it is still degenerating.

One apparently trivial but actually important reason for the failure to realize what changes occur in the topography of tracts at the most cranial levels is the usual practice of dividing the brain from the spinal cord with a sloping diagonal cut. This cut prevents the histological examination of the most cranial segments of the cord. We have always made a practice of leaving the most cranial segments still attached to the medulla oblongata when we remove the brain and cord at postmortem: we divide the cord from the brain by a transverse cut at the junction of the C2 and C3 segments.

In investigating the course and extent of propriospinal fibres and tracts descending from the brainstem, we had to exclude corticospinal fibres from these systems. We therefore studied and present here cases with lesions involving the corticospinal tract situated cranial to the spinal cord. We also studied a large series of cases with motor neuron disease (amyotrophic lateral sclerosis; ALS) in which degeneration of the corticospinal tracts is particularly well seen. Many photographs are included in the hope that they will be useful to neuropathologists and anatomists in the interpretation of degenerating fibres seen in sections of the spinal cord and to draw attention to the characteristic topography of the different spinal segments. Much of our material consists of Marchi preparations as they show degenerating fibres very clearly. It is of course well known that the corticospinal tract consists mainly of small diameter fibres, many of which will not be stained by the Marchi method, but they run intermingled with the larger Marchi-stained fibres.

The features of the corticospinal tracts are considered under the following headings: (1) *Lateral corticospinal tract*: the location and extent in different segments; the passage of the fibres into the grey matter; contralateral corticospinal fibres in the anterior column; the relationship of the denticulate ligament to the tracts in the cervical segments. (2) *Anterior corticospinal tract*: the asymmetry, size, location and caudal extent; the relationship of the tract to other tracts of the anterior column. (3) *Frequency of asymmetry of the spinal cord.*

MATERIAL

The material consisted of 25 cases with supraspinal lesions causing degeneration in the spinal cord limited to the corticospinal tracts and 45 cases of motor neuron disease (ALS). Of the latter cases, 23 were from our own series and 22 were from the Radcliffe Hospital collection, kindly lent by Dr J. T. Hughes for this study. There were 85 cases with anterolateral cordotomies. Control cases without degeneration of the corticospinal tracts consisted of 25 cases with normal spinal cords of which 12 were from nonneurological cases and 13 from neurological cases in which the cord was normal. These were patients with miscellaneous supraspinal lesions, such as cases of closed head injury, and patients who had had stereotactic operations for parkinsonism From this material, the following cases are used for illustrations: (1) 5 cases with cerebral lesions causing marked degeneration in the medullary pyramids, in 1 of which there were bilateral cerebral lesions causing degeneration of both pyramids; in 3 of these 5 cases there were no other lesions, but in 1 there was also an anterolateral cordotomy and in 1 degeneration of the central tegmental tract; (2) 1 case of anterolateral cordotomy, (3) 2 cases with motor neuron disease (ALS) with degeneration of both medullary pyramids.

METHODS

The cords were fixed in formol saline. In all cases the majority, and in some cases all spinal segments, were examined histologically. Each segment was identified and cut transversely just cranial to the insertion of the most cranial nerve roots. This plane is taken arbitrarily as indicating the cranial limit of each segment. Most segments were sectioned in the transverse plane; in a few cases some segments were sectioned in the coronal or sagittal planes.

The segments were mostly divided into several blocks, which were processed for different staining methods. The majority of blocks were embedded in celloidin; some wax-embedded and some frozen sections were also prepared. A wide range of histological methods was applied. Extensive use was made of the Marchi method and of myelin staining, including Weigert-Pal, Loyez, luxol fast blue with cresyl violet, owing to their good photographic properties. Other stains used were phosphotungstic acid haematoxylin, Häggqvist, Gros-Bielschowsky, Nauta, Marsland, Glees and Erikson (original method and modifications), haematoxylin and eosin, haematoxylin and van Gieson, thionin, Victoria blue, and Scharlach R.

OBSERVATIONS

Lateral corticospinal tract

Cases 1, 2 and 3, (figs 1–3, 5–9) show the course and relationships of the corticospinal tracts throughout the spinal cord. In these 3 cases there is clear-cut degeneration in the

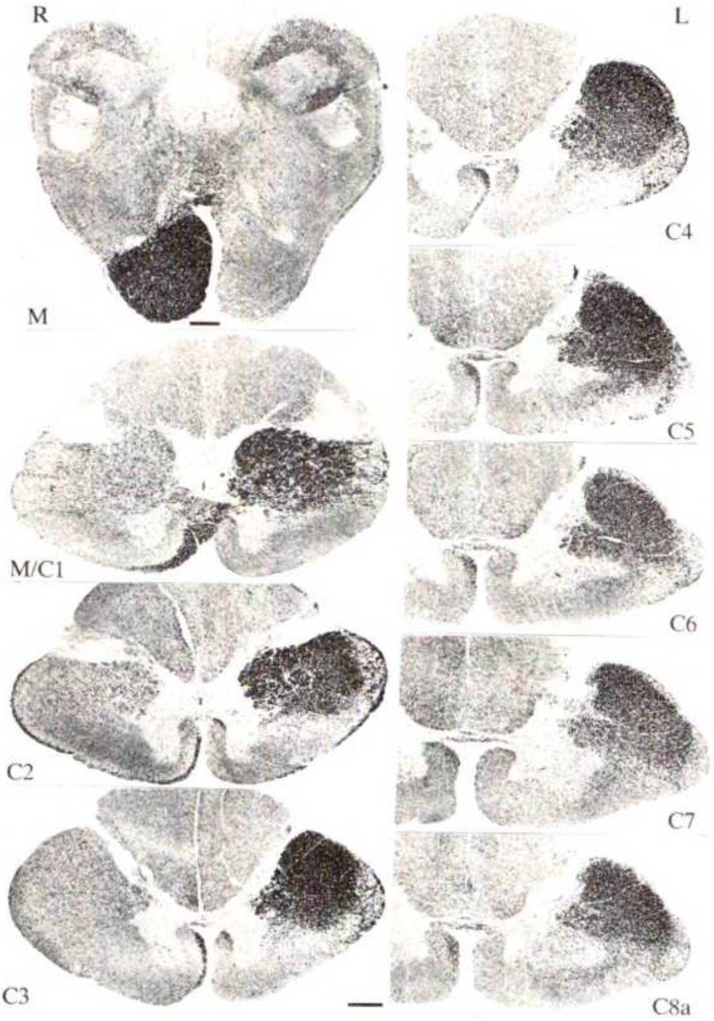

FIG. 1.

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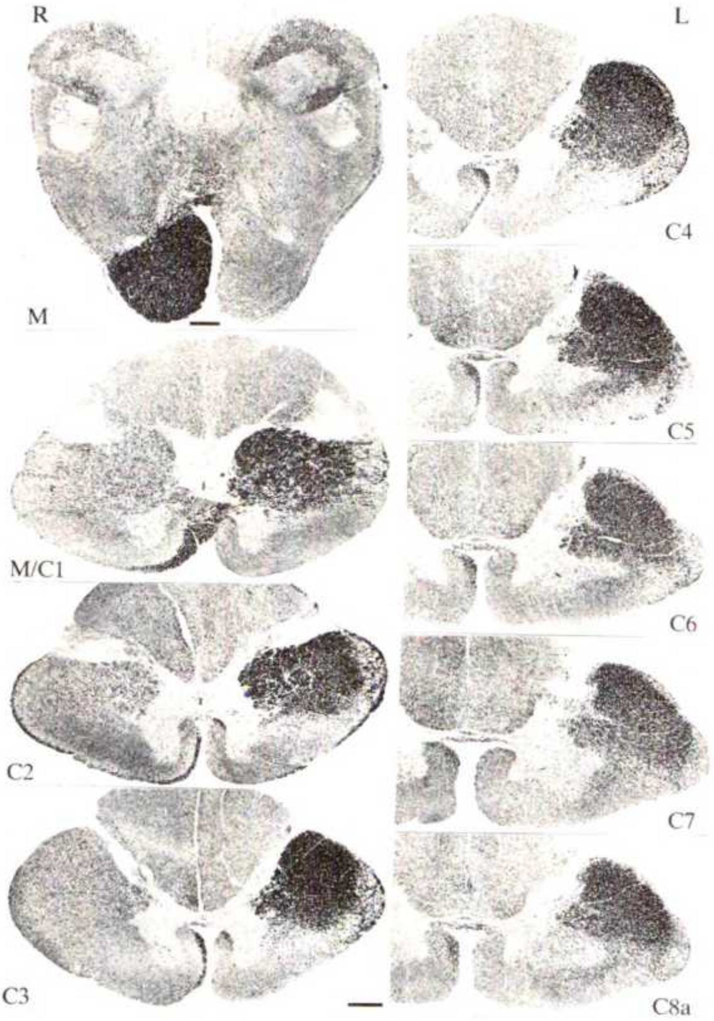

FIG. 1.

corticospinal tract on the left, due to the right cerebral infarct, and degeneration of the spinocerebellar fibres on the right, due to a large cordotomy incision in the upper thoracic cord.

Location and extent of lateral corticospinal tract in different segments

The location of the lateral corticospinal tract is influenced by the size and position of the anterior and posterior horns as well as by the shape of the cord itself. In the first two cervical and the lumbar segments, the large caput of the posterior horn lies, in most cords, lateral to the neck of the horn. In other segments, the caput lies in the same oblique medioposterolateral plane as the neck (fig. 1). In the cervical enlargement, the cord is larger and broader owing to the increased size of the horns, especially the anterior horn. In these segments, the corticospinal fibres are spread throughout a very wide area of the posterolateral and anterolateral columns, and a large number of the fibres lie well anterior to the plane of the central canal.

A further fact affecting the location of the tract is the width of the layer of propriospinal fibres along the posterior horn and around the anterior horn. This layer is much broader in the cervical and lumbar enlargements than elsewhere in the cord. Throughout the cervical and thoracic segments, the tract lies up against the posterior horn, and here is mixed with the propriospinal fibres.

Immediately after the fibres have crossed the decussation to descend in the posterolateral column, some of them lie on the periphery of the cord, as is seen in fig. 1 (M/C1). In the C1 and 2 segments, the tract fills the whole of the posterolateral sector except for a broad peripheral zone occupied by spinocerebellar fibres. Some corticospinal fibres, separated off from the main tract, are scattered on the posterolateral periphery of the cord, and narrow bands of corticospinal fibres lie between the main mass of the tract and the periphery, among spinocerebellar fibres. The number of spinocerebellar fibres along the periphery varies from cord to cord, as can be seen by comparing the cervical segments in the 3 cases illustrated in figs 1, 2, 5 and 6. In the C1 and 2 segments, the outline of the tract on transverse section is approximately rectangular, the long axis lying transversely. Figs 1 and 5 show that, with regard to the anterior extent of the tract in the C1 and 2 segments, many of the fibres lie well anterior to a transverse plane passing through the central canal. Although this is usual, it is not invariable.

In the C3 and upper part of C4 segments (figs 1, 2, 5, 6), the tract fills the posterolateral sector and extends into the anterolateral sector. Posteriorly, it lies along the periphery of the cord. Medially it abuts against the posterior horn.

The Loyez preparation of Case 3, illustrated at higher magnification (fig. 7, C5, C6), shows that the pallor within the area of the tract is not absolute, for some stained myelinated fibres are present. They are more numerous medially. These may be ipsilateral lateral corticospinal fibres or fibres of other systems, such as propriospinal fibres running within the area of the corticospinal tract.

There are often corticospinal fibres lying anterior to the main mass, separated off as islands—for instance in fig. 1 (C4, C5) and fig. 7 (C5). In addition to this, in most cords there are fibres in the cervical segments that are arranged in a continuous band

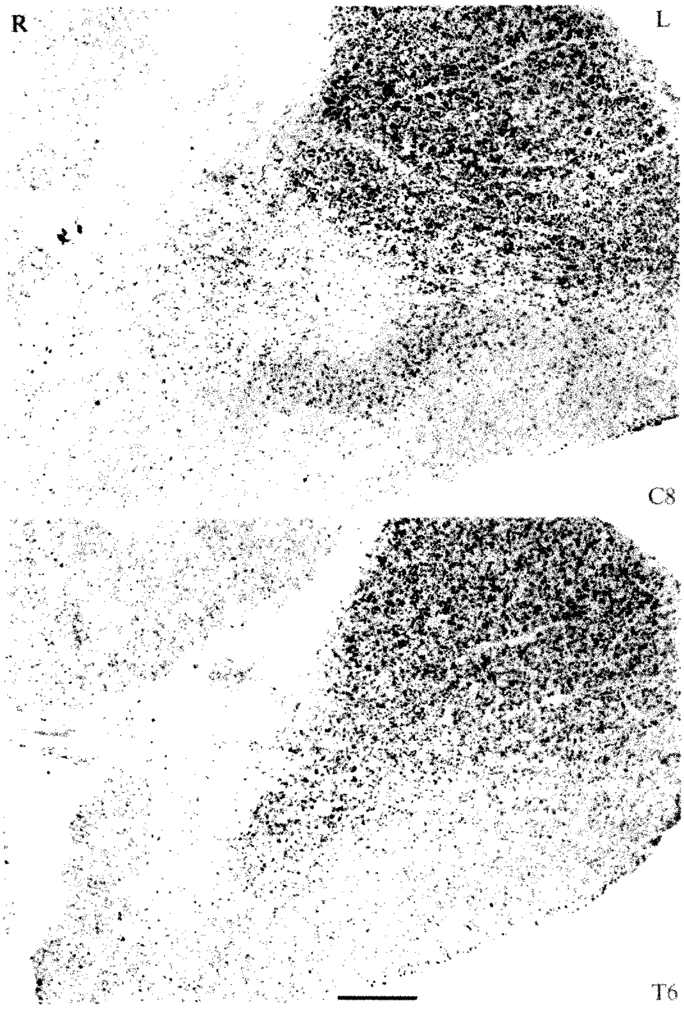

FIG. 3. *Case 1*. Enlargements of C8 and T6 showing numerous degenerating fibres surrounding the entire lateral and anterior borders of the anterior horn in C8 and the lateral border in T6. Marchi preparations. Bar = 0.5 mm.

in transverse sections between the main mass of the tract and the periphery of the cord in the anterolateral sector. These anteriorly running fibres can be seen in figs 1 and 2 (C3), fig. 5 (C3, C4), fig. 6 (C3) and fig. 4 (C2). In figs 1 and 9 (C6, C7), a large amount of the tract is situated anterior to the plane of the central canal. This is partly because many of the fibres move anteriorly to enter the anterior horn.

The relationship of the tract to the spinocerebellar fibres is shown in fig. 6, in which the ascending spinoreticular, spinothalamic and spinocerebellar fibres on the right can be compared with the descending corticospinal fibres on the left in the C1 and C3 segments. Where the lateral corticospinal tract lies along the periphery of the cord, the spinocerebellar fibres are displaced anteriorly: this is seen most clearly in fig. 6 (C3)

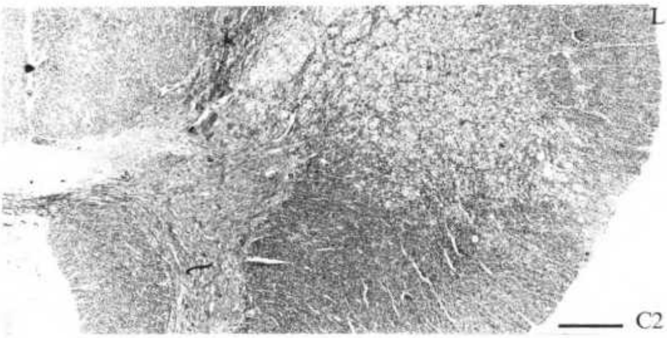

FIG. 4. *Case 4*, male aged 32 yrs. Extensive haemorrhagic necrosis due to stereotactic coagulation of globus pallidus. Much destruction of tissue in the hemisphere extending down into midbrain. Survival: 8 months. Degeneration in right medullary pyramid and right central tegmental tract. In C2, fibres of the lateral corticospinal tract run on the periphery of the anterior part of the lateral funiculus. Silver stain. Bar = 0.5 mm.

by comparing the two sides. In the C4 segment, a few spinocerebellar fibres usually lie between the tract and the periphery (figs 1, 5, C4). In the C5−C7 segments, the spinocerebellar fibres lie in a narrow band along the periphery (fig. 1, C6, C7; fig. 5, C7), the corticospinal fibres being spread over a wide area of the posterolateral sector and extending into the anterolateral sector. Where this tract is large, as in the C8 segment (fig. 10, left), it approaches the periphery of the cord, separating the band of

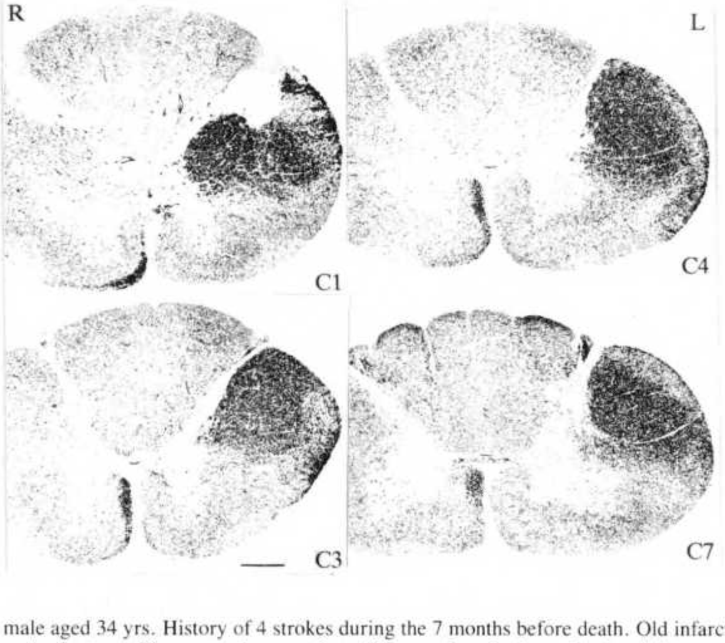

FIG. 5. *Case 2*, male aged 34 yrs. History of 4 strokes during the 7 months before death. Old infarct in distribution of the left middle cerebral artery. There was degeneration of the corticospinal tract in the right pyramid and also of the central tegmental tract. The cervical cord shows the typical location and features of degenerating corticospinal tracts. Marchi preparations. Bar = 1 mm.

spinocerebellar fibres into two groups, one lying in the posterior two-thirds of the periphery of the sector, the other in the anterior third, extending into the anterolateral sector. In the T1 segment the spinocerebellar fibres form a continuous band, peripheral to the tract.

The corticospinal tract remains in the posterolateral sector in the T1 segment, its anterior border lying on the plane or just posterior to the plane of the central canal (fig. 2, T1; fig. 6, T1, left). From T2 to about T7, the outline of the tract on transverse section is triangular, the anterior border of the triangle running slightly posterolaterally from about the lateral horn to the periphery (fig. 2, T6; fig. 8, T4). From about T8 to T12, the anterior border of the tract runs obliquely anterolaterally, in most cords lying anterior

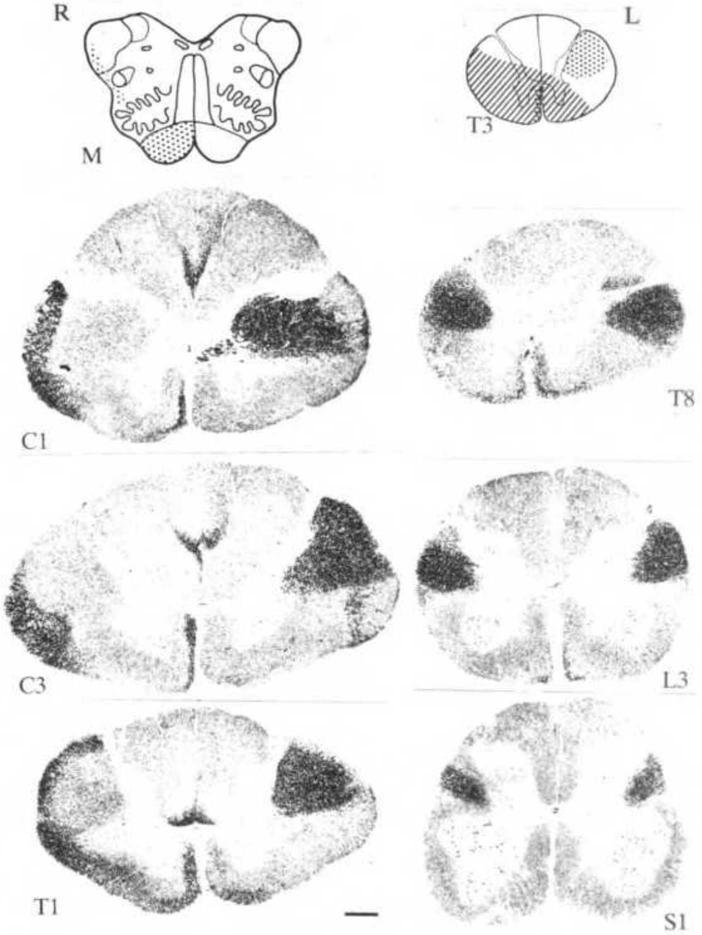

FIG. 6. *Case 3*, female aged 58 yrs. Developed left hemiplegia from infarction in territory of right middle cerebral artery 2 days after an anterolateral cordotomy at T3. Survival: 8 months. Degeneration of corticospinal fibres in right pyramid and also degeneration of right spinorecticular, spinothalamic and spinocerebellar tracts. There is ascending degeneration on the right and descending degeneration on the left. Marchi preparations. Bar = 1 mm.

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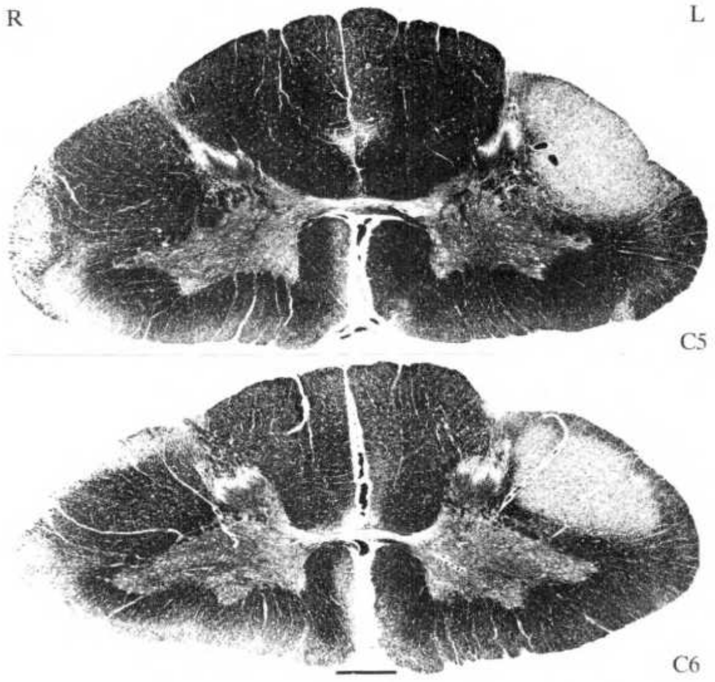

FIG. 7. *Case 3.* Different location and relationships of ascending and descending fibres in C5 and C6. Myelin preparation. Bar = 1 mm.

to the central canal (fig. 2, T11; fig. 6, T8, left). As the cord is descended, the number of spinocerebellar fibres decreases and so the tract comes to lie progressively nearer the periphery. In the lumbar and sacral segments, the tract lies along the periphery of the cord posterolaterally. The anterior boundary of the tract lies at about the plane of the central canal (fig. 2, L3; fig. 6, L3, S1). In the lower thoracic and lumbosacral segments, some of the fibres lie anterior to the main mass, within a peripheral triangular area, intermingled with other fibres (fig. 8, T8, L2).

The corticospinal fibres are mingled with propriospinal fibres near the grey matter and spinocerebellar fibres nearer the periphery. The degenerating corticospinal fibres can be clearly differentiated from the normal fibres medially, anteriorly, and peripherally in the C6 segment in fig. 7.

Passage of corticospinal fibres into the grey matter

In cases in which the period of degeneration was short, fibres of the tract can be seen entering the intermediate zone and the anterior horn. Many of the fibres encircle the greater part of the anterior horn in the cervical enlargement, and in some cords, they can be seen to enter the horn from its anterior border. These features are shown in fig. 1, C7, C8a; fig. 2, C8b; fig. 3, C8; and fig. 5, C4, C7, and in the thoracic cord, fig. 2, T1, T6; and fig. 3, T6.

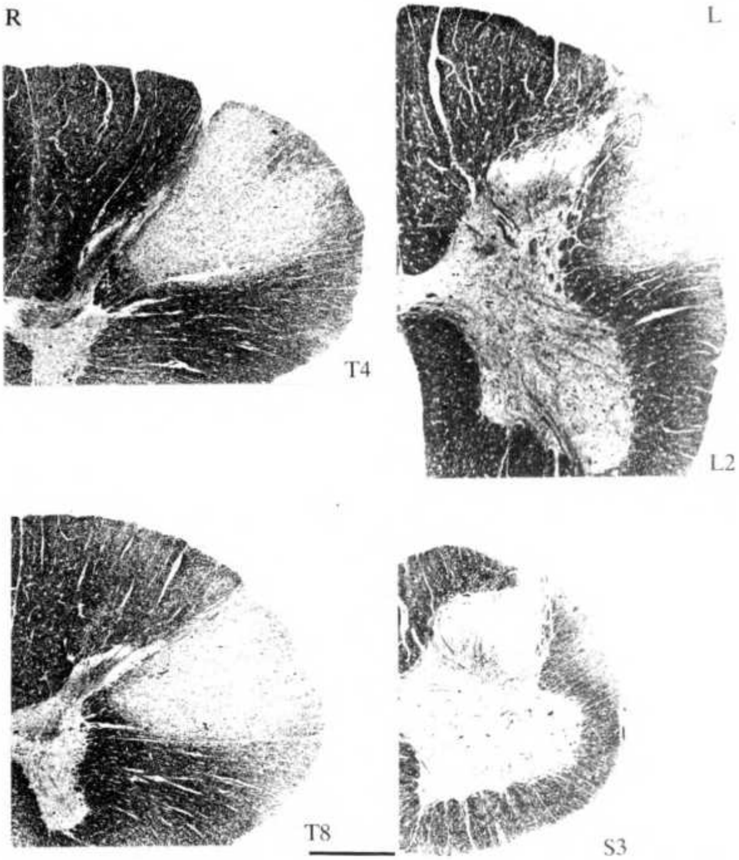

FIG. 8. *Case 3.* Extent and location of lateral corticospinal tract in midthoracic, lumbar and sacral segments. Aberrant fibres from the lateral corticospinal tract are seen on the periphery of the anterior part of the lateral funiculus in T4 and T8. Myelin preparations. Bar = 1 mm.

Contralateral corticospinal fibres in the anterior column

In some cords there is a small bundle of fibres running in the posterolateral part of the anterior column, close to the medial aspect of the anterior horn. These fibres have crossed in the decussation; they are the more caudal fibres of the decussation which are prevented from reaching the posterolateral column by the presence of the anterior horn. In fig. 1 (M/C1), they leave the mass of decussating fibres and turn anteriorly to lie on the medial border of the anterior horn. From there, they can be followed down in this position (fig. 1; fig. 5, C3, C4; the enlarged photograph of C3 in fig. 2) as far as the C6−7 segment; we have not detected them further caudally. In some sections they can be seen passing into the anterior commissure; they have not been seen entering the anterior horn against which they are lying.

This small group of fibres is not Barnes's bundle, for this deeply lying bundle consists of fibres that have crossed to the other side of the cord, whereas Barnes's bundle consists

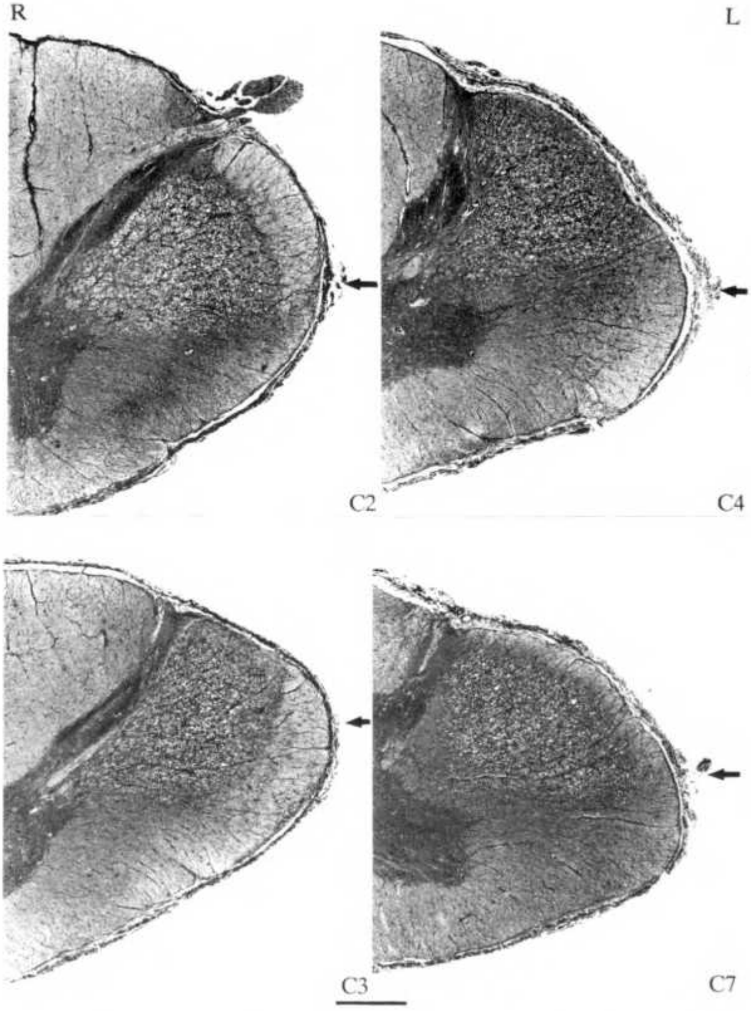

FIG. 9. *Case 3.* The relations of the denticulate ligament to various underlying tracts in the cervical cord. Arrow indicates ligament. Häggqvist preparations. Bar = 1 mm.

of fibres that have not crossed and which continue from the medulla on the anterolateral surface of the cord.

Relationship of the denticulate ligament to tracts in the cervical segments

The clinical importance of the different topography and of the different location of the tracts is obvious when an operation such as anterolateral cordotomy is considered. The denticulate ligament is the main landmark for the operation of percutaneous cordotomy. The relationship between this ligament and the lateral corticospinal tract and spinocerebellar fibres is far from constant. The position of the ligament is shown in one case (Case 3, fig. 9) throughout the cervical cord. In this case, owing to the long survival period, the full anterior extent of the lateral corticospinal tract is not seen. In many cases this ligament was attached to the cord even further posteriorly than in this cord. From fig. 9 and from the Marchi sections of Cases 1 and 2, it can be deduced that an anterolateral cordotomy carried out in the C2 segment, as is nowadays generally done with the percutaneous operation, is likely to involve only the most anteriorly lying spinocerebellar fibres and the most anterior fibres of the lateral corticospinal tract. In the C3 and 4 segments, the incision would be likely to involve all the spinocerebellar fibres as they lie more anteriorly at these levels, as well as the anteriorly lying fibres of the lateral corticospinal tract. When the ligament is attached more posteriorly, more fibres of the corticospinal tract would be damaged. A cordotomy in the C7 to T1 segments would be likely to involve only a smaller number of spinocerebellar fibres that are lying anteriorly and also the aberrant fibres of the lateral corticospinal tract. In view of the more lateral location of the most anterior fibres of the corticospinal tract in the lower cervical segments, these fibres are likely to be involved even in a superficial lesion.

Anterior corticospinal tract

Asymmetry, size, location and caudal extent

The two halves of the cord are commonly asymmetric, and where there is asymmetry, the right half of the cord is usually bigger than the left.

In every example of asymmetry it was observed that the difference in size of the two halves was due to a difference in size of the corticospinal tracts. The size of the lateral tract is reciprocally related to the size of the contralateral anterior tract; for when one lateral tract is larger than the other, it is because more fibres have crossed at the decussation, and so there are fewer fibres in the contralateral anterior tract. This also implies that as the contralateral lateral tract is smaller than its fellow, the anterior tract opposite to this smaller lateral tract is likely to be larger than the opposite anterior tract. The total implication is that the side of the cord with the larger lateral tract will have the larger anterior tract, and so that half of the cord will be larger than the other. Thus it is always found that in such a cord there is a larger number of corticospinal fibres running on one side than the other, and that is usually the right.

This asymmetry with the right side being the larger is shown in 2 cases of motor neuron disease—Case 5 (fig. 10) and Case 7 (fig. 14)—and a case of bilateral cerebral infarctions, Case 6 (figs 11, 12). In Case 5, the areas of pallor devoid of corticospinal fibres are both large on the left, and an area of pallor along the right median fissure normally occupied by the anterior corticospinal tract is absent or minimal. In Case 6, in the cervical cord the right anterior tract is seen to be considerably larger than the left; by the lumbar segments, the left tract is no longer present but the right is still quite large.

It was always seen that a difference in size of the anterior columns was due to a difference in size of the anterior corticospinal tracts. For instance, in fig. 12 (C2) the width of the area of normal fibres between the degenerating corticospinal fibres and

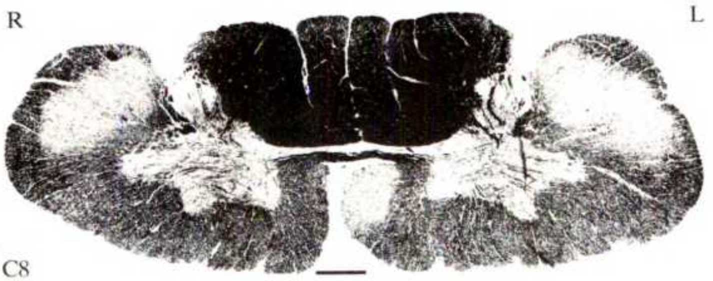

FIG. 10. *Case 5*, male aged 62 yrs. Typical history of motor neuron disease for 2 yrs before death. Marked degeneration of both medullary pyramids. Asymmetry of corticospinal tracts shown in C8. The right lateral tract is smaller than the left; the left anterior tract is large, the right almost absent. Myelin preparation. Bar = 1 mm.

the anterior horns is the same on the two sides. But the right anterior column is larger than the left, as the right anterior corticospinal tract is larger than the left. Other examples are shown in figs 10 and 14. Doubtless the area occupied by the propriospinal fibres is the same on the two sides, and the medial longitudinal fasciculus is a very small tract in comparison with the anterior corticospinal tract.

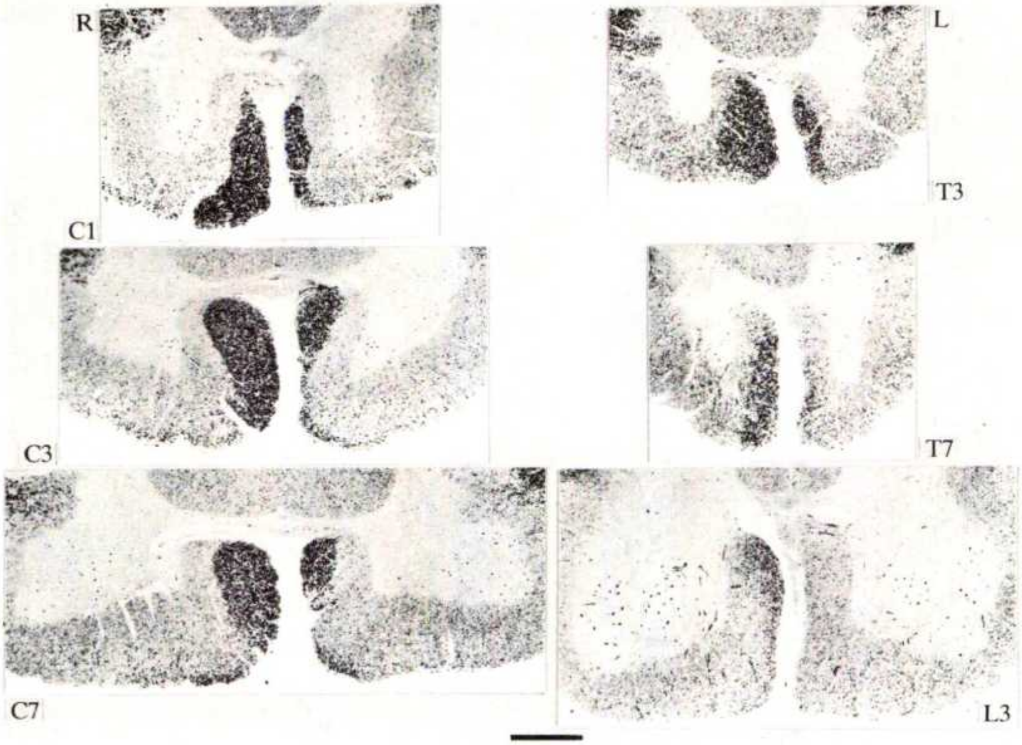

FIG. 11.

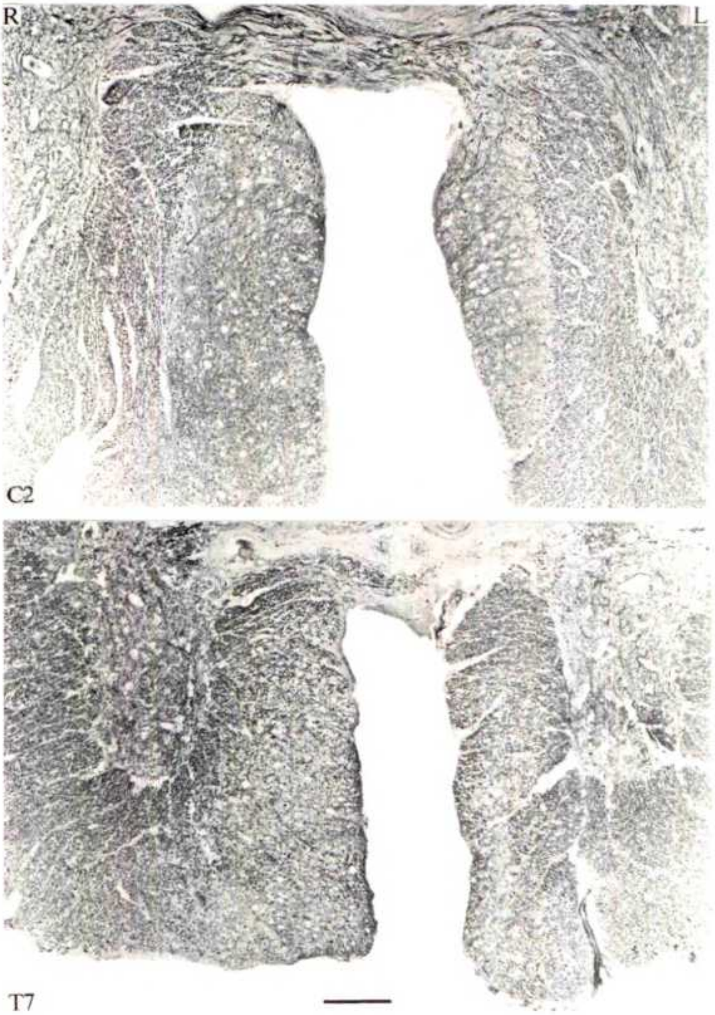

FIG. 12.

FIGS 11 and 12. *Case 6*, female aged 49 yrs. Paraplegic and immobile for 12 wks before death following extensive infarction in the territory of both middle cerebral arteries. There was marked degeneration in both medullary pyramids. In fig. 11, sections show the location and features of the anterior corticospinal tract in an example in which the tracts reach into the lumbar cord. The right tract is larger than the left. Marchi preparations. Bar = 1 mm. Fig. 12. C2: the pale area due to fibre loss is larger on the right. T7: the distinction between the degenerating medial part and normal lateral part of the anterior columns is much less marked at this level. An irregular band of normal fibres lies along the sulcal periphery of the columns, medial to the degenerated fibres. Silver preparations. Bar = 1 mm.

The anterior tract runs throughout its course in the most medial part of the anterior column. It is thus in the most convenient location for its fibres to cross in the anterior commissure and end in the centromedial part of the anterior horn. In the C1 –4 segments, the tract lies along the whole median fissure and usually spreads around the sulcomarginal angle to reach the anterior surface of the cord. These points can be seen in fig. 1 (C3, C4), fig. 2 (C3), fig. 5 (C1, C3, C4), fig. 6 (C1, C3), fig. 11 (C1, C3 bilaterally)

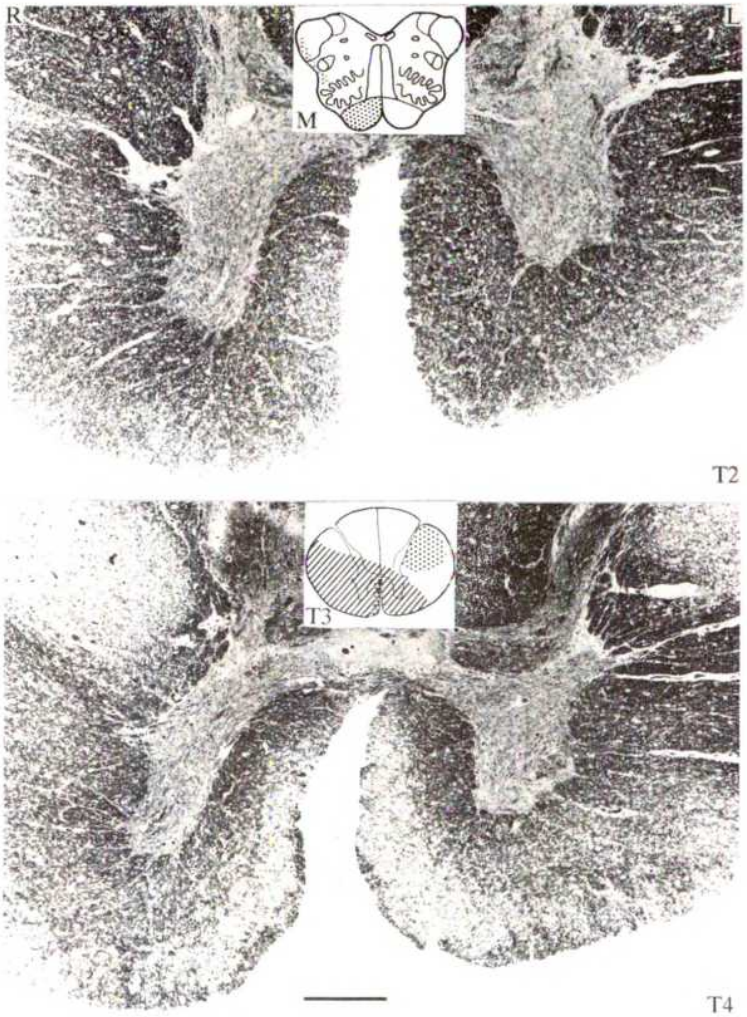

FIG. 13. *Case 3.* Degeneration of right pyramid and right anterior corticospinal tract due to a right cerebral infarct and of tracts in both anterior columns due to a large cordotomy lesion at T3. The pallor in the right anterior column above the cordotomy at T2 along the median fissure is due to the cerebral infarct; the pallor in both anterior columns at T4 is caudal to, and due to, the large cordotomy incision crossing the midline. Normal fibres along the margins of the fissure. Myelin preparations. Bar = 0.5 mm.

and fig. 14 (C3 bilaterally). More fibres are concentrated posteriorly in this area than anteriorly. In some cords, the anteriorly and posteriorly located fibres run separately from each other, this giving the appearance of two separate tracts (fig. 11, C7).

In Case 3, the extent of the anterior tract following a cerebral infarct is shown at T2 (fig. 13). The area of pallor at T4 is larger due to the cordotomy incision at T3, for the incision cut across both anterior columns, thus dividing fibres of groups other than corticospinal. Some of these are fibres of the medial longitudinal fasciculus; others may be vestibulospinal, if this tract is still present at this level.

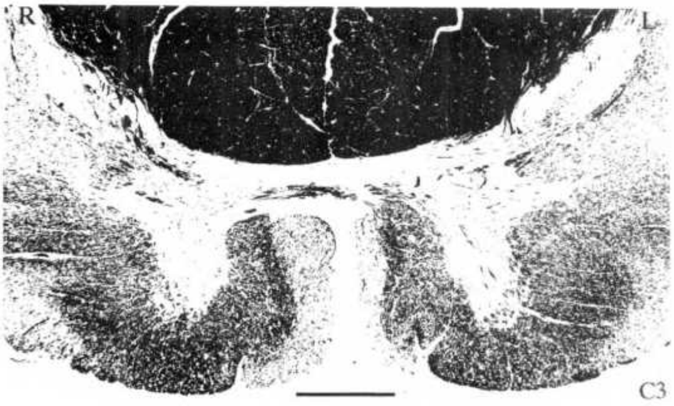

FIG. 14. *Case 7*, male aged 53 yrs. Typical history of motor neuron disease. The anterior corticospinal tracts are asymmetric, the right being larger than the left; shown at C3. Myelin preparation. Bar = 1 mm.

The caudal extent of the tract depends on its size, which reflects the number of fibres within it. When it is small it cannot be identified further caudally than the upper thoracic cord. When it is large it continues into the sacral segments. It then lies along the whole of the median fissure, forming a continuous band (fig. 6, Case 3; fig. 11, Case 6). In the lumbosacral cord, the fibres are usually concentrated in the most posterior part of the fissure (fig. 11, L3).

Relationship of the anterior corticospinal tract to other tracts of the anterior column

In the anterior column are five groups of fibres. They are, lateromedially, propriospinal fibres surrounding the anterior horn, the medial longitudinal fasciculus sometimes called the sulcomarginal bundle when it enters the spinal cord, the commissural fibres, the anterior corticospinal tract, and a narrow band of ascending fibres.

Between the anterior corticospinal tract and the medial border of the anterior horns is a broad zone consisting mainly of propriospinal fibres (fig. 12, C2; fig. 13, T4). There is a group of fibres that runs between the anterior corticospinal tract and the propriospinal fibres. In fig. 14 (C3) they are shown by dark staining immediately lateral to the degenerated anterior corticospinal tract, which is indicated by pallor in this myelin preparation. They are likely to be the medial longitudinal fasciculus.

The group of descending propriospinal fibres termed the commissural bundle by Cajal lies lateral to the medial longitudinal fasciculus and anterior to the commissure. In fig. 4 (C2), fig. 12 (T7) and fig. 14 (C3, left), fibres are seen leaving this bundle and entering the anterior commissure. In fig. 14 fibres from the anterior horn are also seen entering the commissure. Fibres from the anterior corticospinal tract can be seen entering the commissure in fig. 1 (C5, C7, C8a). Considering the fact that the fibres of this tract run to the contralateral anterior horn, it is surprising that this feature is not seen more often.

The anterior corticospinal tract remains separate from other groups in the cervical

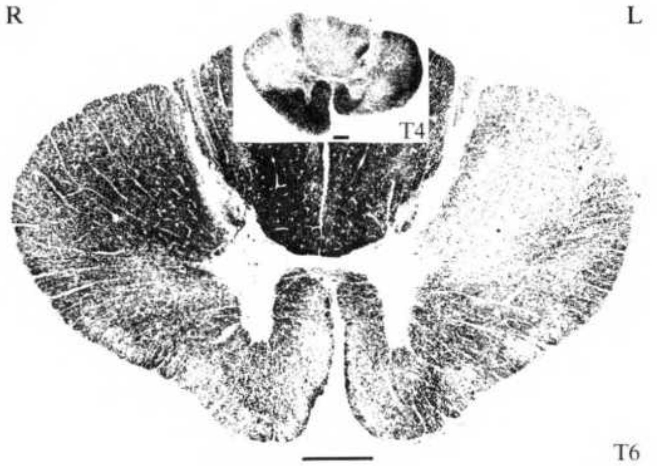

FIG. 15. *Case 8*, female aged 70 yrs. Bilateral anterolateral cordotomy at T5. Survival: 101 days. Section at T4 is in the segment above the surgical incision. Marchi preparation. Bar = 1 mm. T6 (myelin preparation) is in the segment below it. Owing to the pallor in this section in the anterior columns, a narrow irregular band of normal fibres can be seen lying along the medial border of the median fissure. The pallor in the area of the left lateral corticospinal tract in T6 is due to the division of this tract in the cordotomy incision. Bar = 1 mm.

segments, for example fig. 12 (C2); but in the thoracic segment (fig. 12, T7) it becomes more intermingled with the adjacent fibres.

Along the medial border of the median fissure is a very narrow irregular layer of normal fibres (fig. 13, T4; fig. 15, T6). These fibres can be seen more clearly in myelin preparations in cases in which the anterior corticospinal fibres are degenerate. They are ascending fibres. They are seen caudal to the cordotomy incision in figs 13 and 15. The descending fibres, cut by this incision, are degenerating and show as pallor; but ascending fibres remain intact. In fig. 13 they cannot be seen in the T2 segment, as they would have been divided by the cordotomy incision at the T3 segment.

A note on frequency of asymmetry of the spinal cord

As we found asymmetry so frequently in the spinal cords reported here, we examined a series of cords to find out how frequently it occurred. Asymmetry of the cord occurred in 37 of 50 (74%) normal cords; and in 16 of 22 (73%) cases of motor neuron disease. Thus symmetric cords are in the minority. Of our 53 asymmetric cords, the right side was larger in 40 (75%) and the left in 13 (25%). Thus in most cords more corticospinal fibres run on the right side.

DISCUSSION

We have presented evidence as to the extent and location of the corticospinal tracts in the different segments throughout the spinal cord. This was done originally as we found that we needed to have this knowledge in order to establish the anatomy of the tracts originating in the brainstem and of propriospinal fibres, and to correlate motor defects with division of descending fibres.

The increasing proportion of the white matter occupied by corticospinal fibres during evolution of the primates is clearly illustrated by Kuypers (1981). Associated with the great increase in the number of corticospinal fibres in man is the great increase in the area within which fibres of the lateral corticospinal tract lie, and also the spread of the fibres of the tract anteriorly so that they in fact do not remain in the posterolateral sector of the cervical cord.

The topographical arrangement in the cervical cord is such that any segment differs from the next segment and so it can be identified. The arrangement is more constant throughout the thoracic segments, for once the fibres that supply the motoneurons and the interneurons connected to motoneurons of the upper limb have left the tract, the remaining fibres of the tract continue within the lateral columns.

The aberrant groups of fibres running anteriorly to the main body of the lateral tract in the cervical cord have been observed by early workers on this tract. One group of these fibres running on the surface of the cord in the C7 and C8 segments was discussed by Stewart and Turner (1900) and labelled 'Tract 'X', ventro-external to the crossed pyramidal tract'. They raised the question as to whether these could be corticospinal fibres as the fibre spectrum was the same as the main mass of the fibres in the lateral tract, but they could not know this as theirs was a case of a traumatic cord lesion at the C6 segment.

Gray's Anatomy (Williams and Warwick, 1980), curiously, states that the anterior corticospinal tract 'cannot be traced below the middle of the thoracic region'. That it may reach the sacral segments has been known for many years; examples illustrating this are given above. In some textbooks of anatomy it is stated that the fibres of this tract are intermingled with fibres of other descending tracts. Our evidence shows this occurs only in some cases and in some segments of the thoracic cord; in the rest of the cord, the tract remains separate from other descending tracts.

The small group of contralateral corticospinal fibres in the anterior column running along the medial aspect of the anterior horn are fibres that have not decussated and which pass down from the medulla on the anterolateral surface of the cord. These fibres are those illustrated by Collier and Buzzard (1901) in their figs XX and XXII and were named the ventral crossed bundle of the pyramid.

That the commonly occurring asymmetry of the cord is due to the size of the lateral corticospinal tract does not seem to have been stated before; nor that the anterior tract is larger on the side where the lateral tract is the larger. This asymmetry means that there is a larger total number of corticospinal fibres in the larger half of the cord than in the other.

It is surprising that there is a reciprocal relationship in size between the lateral and the anterior corticospinal tracts. For fibres of the corticospinal tracts have different origins, different terminations and different functions. If man is similar to the macaque—and with respect to this question, it is likely to be so—area 4, the somatic sensory cortex, area 5, the second somatic sensory cortex and the supplementary motor area project their corticospinal fibres to end in different laminae of the spinal grey matter (Coulter and Jones, 1977; Murray and Coulter, 1981; Cheema *et al.*, 1984). Furthermore, the fibres of the lateral and the anterior tracts are concerned with different parts of the musculature, the lateral tract with the limbs and the anterior tract with the axial and girdle-to-limb musculature. It is to be noted, however, that in those rare cases in which the entire tract crosses to the posterolateral sector or in which it remains uncrossed in the ventral column, there is no abnormality in function. Presumably the fibres reach their destined targets whatever course they take.

Among the anatomical facts described here, from a theoretical and not a practical point of view, the ascending fibres running along the border of the anterior median fissure are interesting. They have been seen in other anterior cordotomies in the thoracic cord, but we have not illustrated other examples as this paper is concerned with descending and not ascending fibres. They extend for only 2 or 3 segments; accordingly they would seem to belong to the propriospinal system.

The common concept of the spinal cord, learned from anatomical diagrams, is that it is symmetric. In the majority of cords examined by us and reported in the literature, symmetry is the exception, not the rule. In asymmetry, both the anterior and the lateral corticospinal tracts are larger on one side than the other, and it is this that makes one side of the cord larger than the other. Further, the side that is larger is the right: this is so in three-quarters of spinal cords. The literature on this subject up to 1963 was reviewed by Nyberg-Hansen and Rinvik (1963). Since that time, Yakovlev and Rakic (1966) studied the decussation and the distribution of corticospinal fibres in the two sides of the cord in fetuses and neonates, using Fleschig's myelogenetic material. They found that 'More fibres of the left pyramid cross to the right side of the spinal cord than fibres of the right pyramid cross to the left side' and that 'more fibres of the right pyramid descend directly into the right side . . . than fibres of the left pyramid descend into the left side and, therefore, the right side of the spinal cord . . . receives more pyramidal tract fibres from both cerebral hemispheres than the left side'.

Our observation that of asymmetric cords the right side is bigger according to a ratio of 3:1 raises the question whether this is related to handedness. However, the usual ratio given for right to left handedness is 9, 10 or 11 to 1. The relation of handedness to the corticospinal tract was considered by Kertesz and Geschwind (1971). They studied which tract crossed over to the opposite lateral column at the highest level, but found no relationship between this crossing and handedness. It appeared to be significant that, of the brains they dissected, 73% had the crossing from left to right at the highest level and that of our asymmetric cords, 75% had the right side larger than the left and that this asymmetry was always due to a larger number of corticospinal fibres on that side.

In an important paper, Kameyama *et al.* (1963) related the clinical findings of hemiplegia to the variations in the crossing of the fibres at the decussation found postmortem. They found that in those cases in which the hemiplegia was on the same side as the lesion in the internal capsule, it was because there was a large proportion of uncrossed fibres of the corticospinal tract. The manifestations of hemiplegia were less in those cases in which there was more ipsilateral innervation by corticospinal fibres.

In cases with a large proportion of fibres in the anterior corticospinal tract, 'hemiplegia, tendon hyperreflexia, the Babinski reflex may appear separately on the opposite and the same sides'. They proposed that the presence of many uncrossed fibres of the corticospinal tract was a cause of ipsilateral hemiplegia following capsular lesions. This hypothesis provides a cause for this syndrome, probably commoner than that put forward by Kernohan and Woltman (1929), which became well known as Kernohan's notch. But Kernohan's notch remains a likely cause of ipsilateral hemiplegia in some cases of tumours and abscesses.

As the fibres of the lateral corticospinal tract are spread so extensively in the cervical cord, any lesion, surgical or pathological, may damage many of its fibres. Making the denticulate ligament visible in the operation of percutaneous cordotomy is clearly an important guide. Cutting spinocerebellar fibres seems to have no clinical effects but the same cannot be said of corticospinal fibres. At all levels fibres of the lateral corticospinal tract are likely to be damaged by a lateral lesion, in the C3 and C4 segments by a posterolateral lesion, and between C3 and T1 segments by an anterior lesion and certainly by an anterolateral lesion. Conversely, a lesion in the cervical cord that obviously affects the fibres of the posterolateral region will spare the fibres of the tract that are running anterior to the plane of the central canal.

In the recently introduced operation of dorsal root entry zone (DREZ) coagulation by Nashold and Ostdahl (1979), the tract is in more danger. In this operation multiple lesions are made 2 mm apart with a thermal electrode craniocaudally in the posterior horn. In the C3 and C4 segments, the lateral corticospinal tract lies along the posterior surface of the cord. At all levels it lies in contact with the posterior roots as they enter the cord. So far, 2 cases have been studied postmortem. The patient studied by Richter and Schachenmayr (1984) died 5 days after the operation. During those 5 days, 90% of the patient's pain was removed and there was no weakness in his lower limb. The surgical lesion which destroyed laminae I – V of the posterior horn also caused some damage in the adjacent posterior column. The second case was reported by Iacono *et al.* (1988). In this case lesions were made bilaterally in the L2 segment. The one on the left was large and involved the entire posterior horn down to and including lamina VI. The one on the right was smaller, was slightly more lateral and would have damaged many corticospinal fibres. The operation not only removed all pain but caused no motor disability. The reason why there was no motor disability might have been due to the fact that only a certain proportion of the fibres were damaged; and due perhaps to the fact that by the L2 segment, many of the fibres for motility of the lower limbs would have entered the grey matter. One must be aware that lesions made in the future may not always be so well defined and well placed.

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RESPONSE PROFILES OF HUMAN MUSCLE AFFERENTS DURING ACTIVE FINGER MOVEMENTS

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SUMMARY

Twenty-five human muscle afferents from the extensor digitorum muscles of the forearm were studied with the microneurographic method. Single unit impulses were recorded while the subjects performed alternating movements of moderate speed at the appropriate metacarpophalangeal joint. For comparison, responses to imposed movements of similar amplitudes and velocities were also studied.

Most spindle afferents ($n = 17$) provided a stretch response with both kinds of movement. However, the impulse rate was slightly higher and the interspike interval variability much larger during active movement. Two units provided deviating response profiles: a flat profile and a converse stretch response. Small and constant torque loads usually failed to modify the response profile but gave rise to a moderate increase of impulse rate in 50% of the spindle afferents. In one single unit, a converse stretch response appeared with opposing loads.

Tendon organ afferents ($n = 8$) were totally unmodulated by imposed stretch in the relaxed muscle. In contrast, their impulse rate was highly modulated during active movements, often following the rectified EMG which resulted in a converse relationship to muscle length and velocity.

The findings support the view that, in general, human muscle spindles monitor muscle length and velocity in routine movements of moderate speed as long as opposing loads are small, whereas Golgi tendon organs monitor the amount of muscle recruitment. The significance of the deviating response profiles from spindle afferents remains obscure.

INTRODUCTION

For an understanding of the role of muscle spindles in motor control and kinaesthesia it is essential to define the information supplied by these complex sense organs during natural movements. Although muscle spindles are basically stretch receptors, their afferent firing is not always an overt stretch response because the discharge is also dependent on fusimotor activity.

It has often been reported that the individual spindle afferent provides a description of the gross length changes of the muscle in studies of natural routine and unopposed movements in behaving animals (Prochazka *et al.*, 1979; Taylor and Appenteng, 1981; Larson *et al.*, 1983; Loeb *et al.*, 1985). On the other hand, a number of investigations

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with conscious human subjects as well as anaesthetized and decerebrate animals have demonstrated that spindle afferent rates may be poorly related to gross movements but highly dependent on the amount of muscular contraction (Critchlow and von Euler, 1963; Severin *et al.*, 1967; Vallbo, 1973, 1974; Burke *et al.*, 1978*a*, *b*; Hulliger *et al.*, 1982, 1985). In this latter group of investigations, very slow movements have mostly been studied, whereas moderate velocities were analysed in the former, and it has been suggested that speed is an essential factor which determines the response profile of spindle afferents in conscious human subjects as well as in behaving animals (Prochazka *et al.*, 1979). However, only a limited amount of data are available from human subjects on movements above very low velocities. These data actually suggest a variety of response patterns but are difficult to interpret because of the lack of more systematic descriptions (Burke *et al.*, 1978*b*). Moreover, unit identification was based on a single test which later was demonstrated to give imprecise identification in a considerable number of units (Edin and Vallbo, 1987).

The present study was designed to explore the question as to whether human spindle afferents uniformly provide a clear stretch response during active movements of moderate speed as found in behaving animals. It will be shown that most of them did but a minority deviated, producing a converse stretch response or a flat response. Tendon organ afferents, on the other hand, provided a response related to the amount of EMG of the parent muscle.

METHODS

Data were collected in 14 experiments on 14 healthy subjects, 6 males and 8 females, aged 22–36 yrs. The subjects were medical students or medical personnel. All gave informed consent before the experiment, according to the Declaration of Helsinki. During the experiment, the subject was seated comfortably in a dentist's chair. His/her left forearm lay in an adjustable vacuum cast and the hand was stabilized with a mechanical support. The wrist joint was held at a semiflexed position. The arrangement provided firm fixation of the forearm and hand, but still permitted free finger movements.

Nerve and EMG recordings

The microneurographic method of Vallbo and Hagbarth (1968, for details, *see* Vallbo, 1972) was used to record impulse activity of 25 single afferent units from the left radial nerve. Insulated tungsten needle electrodes, with an impedance of 200–300 kΩ measured at 1 kHz and with a tip diameter of about 5–10 μm, were inserted percutaneously 6–8 cm above the elbow. Nerve fascicles innervating the finger extensor muscles were searched for, while the relaxed fingers were continuously flexed and extended by one of the experimenters. A multiunit response to these stimuli indicated that the electrode tip had attained an intrafascicular position. Minute adjustments of the electrode were then made until a single afferent was isolated.

The unit was tested for its responsiveness to passive and active movements of individual fingers so that the receptor-bearing portion of the muscle was identified. The particular finger which elicited the best movement response was referred to as the optimal finger and it was assumed that it was operated by the receptor-bearing portion of the muscle. Further assessment of the location of the ending was accomplished by checking its response to local pressure.

Four silver disc electrodes (SLE, B19) were fixed in suitable positions on the skin over the finger extensor muscles in order to allow optimal recordings from the separate portions of the muscle. On isolation of a single afferent unit, the pair of electrodes which gave the best recording of the EMG activity of the parent muscle portion was selected.

Actuator

The optimal finger was strapped with a splint which permitted movements only at the metacarpophalangeal joint. The splint was attached to the spindle of an actuator by means of a hinged bar of low mass (Edin and Vallbo, 1987). Basic features of the actuator have been described in previous studies (Hulliger *et al.*, 1982; Al-Falahe and Vallbo, 1988). In the present study, it was used to impose movements of desired amplitudes, speed, and acceleration and to oppose active movements with constant torque loads. The angular position, speed of movement, and torque were continuously recorded. The actuator was controlled by a microcomputer which was programmed to produce test sequences and to command other laboratory devices, for example, a tape recorder, display units, and auditory cues to the subject.

Unit classification

Twenty-five units were studied and subjected to a series of 3–5 identification tests (Table 1).

Twitch test. Maximal twitch contractions were produced by stimulation through a bipolar cushion electrode (Disa, 13k62) at the motor point of the receptor-bearing portion of the muscle (Edin and Vallbo, 1987). Successful twitch tests were pursued with 19 units either under isometric conditions (n = 12), or during active muscle lengthening (n = 7), introduced in order to produce a background discharge.

Ramp test. Ramp and hold movements were imposed at the metacarpophalangeal joint of the optimal finger while the subject was instructed to remain relaxed. A standard amplitude of 20° was used with two stretch velocities, 20 deg/s and 50 deg/s. A stretch response suggested a spindle origin for the discharge, whereas a lack of stretch response suggested a tendon organ origin as it has been found that most spindles from these muscles exhibit a distinct response to imposed stretch whereas tendon organs do not (B.E. and Å.B., unpublished). Furthermore, the classification of units as primary spindle afferents was supported if the unit exhibited one or more of the following criteria: initial burst, high dynamic index, deceleration response, and silence during the main part of imposed muscle shortening (Matthews, 1972). High dynamic index was defined as an index larger than the difference in sustained discharge at the two hold phases (Matthews, 1972).

Contraction-relaxation test. Subjects were instructed to perform slowly rising isometric contractions and then relax as quickly as possible. The peak torque was 0.1 Nm, corresponding to 11–23% of the maximal voluntary torque of the finger's extensor muscles.

A prominent increase of the discharge accompanied the abrupt fall in tension at the end of the contraction in 11 units. This was taken as a strong support for the unit being a spindle afferent rather than tendon organ afferent. A decrease of discharge or a lack of impulse acceleration during the contraction phase also suggested muscle spindle origin.

Additional tests. For 6 units (unit numbers 5, 6, 10, 12 in the spindle group; 7, 8 in the Golgi group), two additional identification tests were conducted: stretch sensitization and small sinusoidal joint movements at 50 Hz superimposed on ramp and hold stretch. Three units (unit numbers 5, 6, 10 of the spindle group) exhibited stretch sensitization of the type characteristic for muscle spindle primary afferents. Moreover, the discharge of all 4 spindle units was locked to the 50 Hz sinusoids, further supporting a muscle spindle origin (Matthews and Stein, 1969).

Unit sample. The outcome of the test battery is shown in Table 1 for the total sample of 25 units; 17 units were identified as spindle afferents, 8 as primaries and 1 as a secondary on the basis of these tests. In addition, it seemed reasonable to differentiate another 6 spindle afferents as primaries and secondaries when all available observations were considered, including active movement data described in the Results section. The test battery identified 4 of the other units as Golgi tendon organs. Moreover, 4 additional units were found to have the same response properties as the identified tendon organ afferents although the twitch test was not successful. As will be described in detail in the Results section, these 8 units constitute

TABLE 1. IDENTIFICATION DATA

Unit no.	*Unit type*	*Twitch test*	*Response to ramp stretch*	*Initial burst*	*High dynamic index*	*Deceleration response*	*Stop during shortening*	*Isometric contraction*	*Relaxation burst*
A. Muscle spindle afferents									
1		++	+	−	/	/	/	+	−
2	(IA)	/	+	−	/	/	/	−	+
3	IA	++	+	−	+	+	+	−	+
4	IA	++	+	+	+	−	+	−	+
5	IA	++	+	−	+	−	+	+	+
6	IA	++	+	−	−	−	+	+	+
7	(IA)	+	+	−	−	−	/	+	/
8	(IA)	/	+	+	+	/	/	+	+
9	IA	++	+	+	+	+	+	+	+
10	IA	++	+	+	+	+	+	+	−
11		+	+	−	−	−	+	−	−
12		+	+	+	−	+	+	−	+
13	(II)	+	/	/	/	/	/	−	−
14	II	+	+	−	−	−	−	−	+
15	IA	+	+	+	+	−	+	+	/
16	IA	++	+	+	+	/	+	−	+
17	(IA)	+	+	−	−	−	+	+	+
B. Golgi tendon organ afferents (identified and provisional)									
1	(IB)	/	−	/	/	/	/	+	−
2	IB	−	−	/	/	/	/	+	−
3	IB	−	−	/	/	/	/	+	−
4	(IB)	/	−	/	/	/	/	+	−
5	(IB)	/	−	/	/	/	/	+	−
6	(IB)	/	−	/	/	/	/	+	−
7	IB	−	−	/	/	/	/	+	−
8	IB	−	−	/	/	/	/	+	−

Identification data of 17 units classifed as muscle spindle afferents and 8 units classified as Golgi tendon organ afferents. *See* text on notation concerning unit type in column 2. The plus signs signify, in column 3, unloading response to electrically-induced muscle twitch, in column 4, a clear stretch response to imposed ramp movements, in column 5, a prominent initial burst, in column 6, a dynamic index larger than the difference in impulse rate at the two hold phases, in column 7, a clear deceleration response at the end of the ramp stretch, in column 8, silence during substantial part of imposed muscle shortening, in column 9, enhanced discharge during isometric contraction, and in column 10, a burst of impulses when the subject promptly relaxed after an isometric contraction The double plus signs in column 3 denote an unloading response to twitch and, in addition, acceleration during the falling phase of the twitch. Minus signs in column 3 indicate an accelerated discharge on the rising phase and close to the peak of the electrically-induced muscle twitch. Minus signs in the other columns indicate lack of the features described above, whereas a solidus indicates that the test was not conducted or the feature could not be assessed. This was, for instance, true for all the Golgi tendon organ afferents with regard to details of ramp response because they did not respond to stretch Four of the latter were only provisionally classified because the twitch test was lacking. These units are indicated with parentheses around IB. Two extra tests were performed with 6 units: 5, 6, 10 and 12 from the spindle group as well as 7 and 8 from the tendon organ group (*see* text).

a very homogeneous group consistent with their all being Golgi tendon organs and they will be presented as such.

Experimental test procedures

A series of pseudosinusoidal movements were imposed on the optimal finger while the subject was instructed to remain relaxed and note movement parameters in order to reproduce similar movements shortly afterwards. The imposed movements which were of constant and identical acceleration and deceleration were executed around the resting position of the finger. Their amplitude was 25° with maximal and minimal velocities of 20 deg/s and −20 deg/s, respectively. The cycle duration was 5 s.

Series of active movements intended to mimic the imposed movements were first executed with no external load and with small constant torque loads opposing the finger extensor muscles. The size of the load was roughly scaled according to the strength of the subject's finger muscles and varied between 0.05 Nm and 0.12 Nm, corresponding approximately to 5–15% of maximal voluntary torque of the finger's extensor muscles. Nine spindle afferents and 2 tendon organs afferents were tested in two or more series with opposing loads of varying sizes differing by a factor of about 1.5. The individual series of movements, imposed as well as active, consisted of at least 3 full cycles, usually lasting 15–25 s. Although it was pointed out to the subject that he should produce movements of a similar time course to the imposed movements, no particular measures were taken to have him make identical movements. Visual clues were absent during imposed as well as active movements.

Relative movement amplitude and velocity

The amplitude of the pseudosinusoidal movements corresponded to 2–2.5% relative stretch of the muscle fibres and velocities of approximately 1.5–3%/s.

Data collection and analysis

A Philips analogue tape record (Analog 714) was used to record the signals, namely nerve activity, EMG activity, angular position at the metacarpophalangeal joint, angular velocity, and torque. EMG activity was recorded either directly or after rms processing in a hardware unit. The signals were subsequently displayed on paper with a Gould (ES 1000) electrostatic recorder for detailed examination and measurement.

The mechanical signals—angular position, angular velocity, and torque signals, as well as the rms-processed and low-pass filtered (47 Hz) EMG signal—were sampled with a minicomputer (Nord 500S) with a sampling rate of 100 Hz. The EMG signal was further processed by calculating the moving average of 11 bins corresponding to low-pass filtering at about 4 Hz. Single unit impulses were retrieved from microneurography records using a microprocessor-based device as previously described (Edin *et al.*, 1988). This procedure includes inspection of the shape of individual nerve impulses. The times of occurrence of spikes were stored in the computer. Plots of the sampled signals versus time were produced with a laser printer.

Linear regression analyses were obtained between instantaneous impulse rate on the one hand, and joint position, angular velocity, as well as (filtered) EMG, on the other. Bin width was 10 ms. Bins which did not contain a nerve impulse were discarded; data from phases of afferent silence were therefore not considered.

Conventional parametric statistical methods were used in the analysis.

RESULTS

Test movements

Muscle afferents from the finger extensor muscles were studied with single joint movements which were restricted to the metacarpophalangeal joint of the optimal finger (*see* Methods) (fig. 1). First, a series of pseudosinusoidal movements were imposed by an actuator while the subject was instructed to remain relaxed. The subject was then requested to perform actively a number of similar movements, unopposed as well as opposed by small and constant torque loads. It was found, in agreement with previous studies (e.g., Hulliger *et al.*, 1985), that the general response characteristics of the individual spindle afferent were highly uniform when a test was repeated several times. This was true for active as well as imposed movements. The only notable exception was that some units exhibited a substantial variability when the subject was unable consistently to keep his muscles relaxed with imposed movements (*see below*).

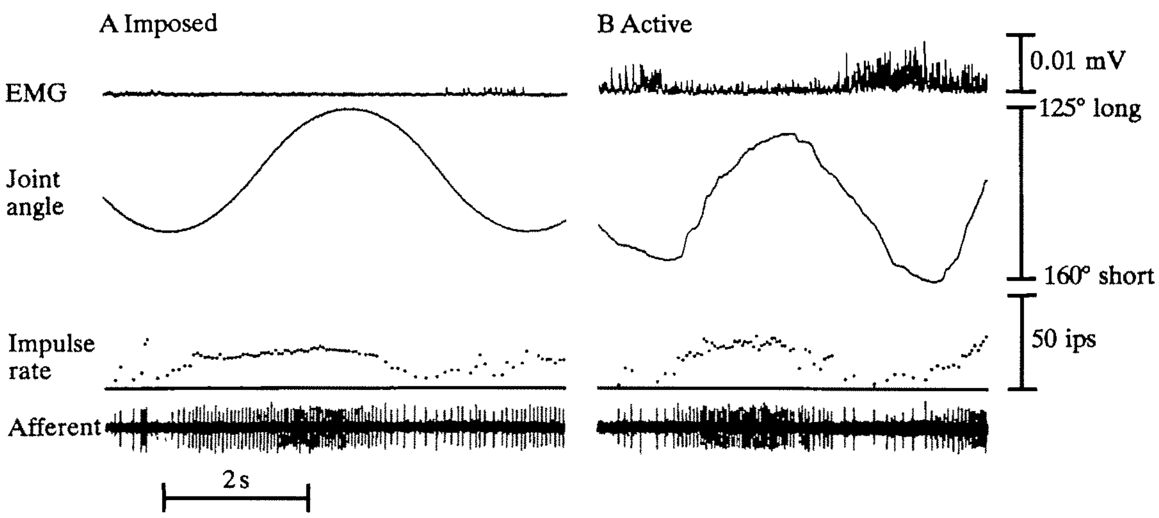

Fig. 1. Spindle response to the two kinds of finger movements studied (unit 12). A, imposed pseudosinusoidal movements, and B, active movements, mimicking the imposed movement, with a small torque load opposing the extensor muscles. Traces from above show rms-processed EMG, metacarpophalangeal joint angle of the test finger, instantaneous impulse rate of a spindle afferent from the extensor muscle, and the original impulse record. Flexion which implies stretch of the parent muscle is indicated upwards in this and all the other figures. (ips = impulses per second)

Muscle spindles

Imposed movements

A sample run from the response of each of the spindle afferents to imposed movements is shown in fig. 2. The majority provided a stretch response. Many units exhibited a relatively high dynamic component, evident from the impulse rate being high during

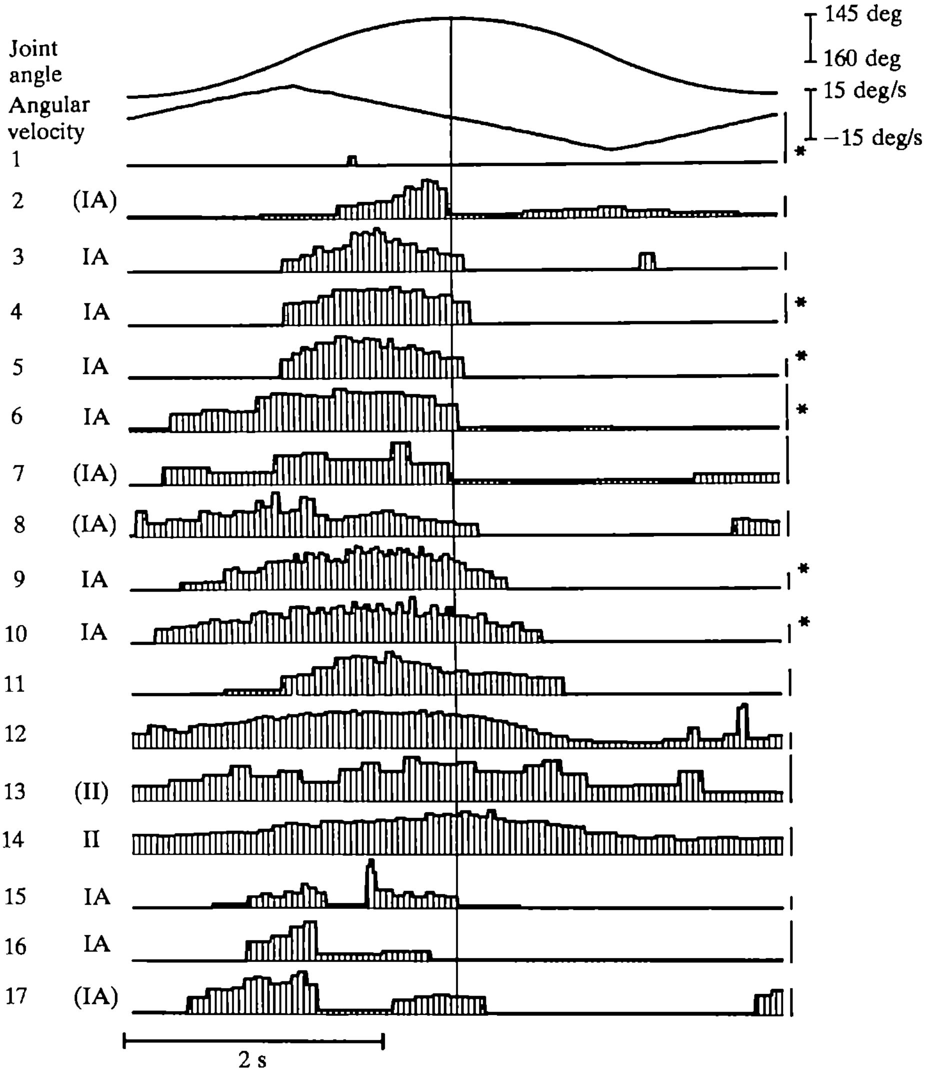

FIG. 2. Spindle response to imposed pseudosinusoidal movements Individual sample runs from all the studied spindle afferents Records, from above, show metacarpophalangeal joint angle of the test finger, angular velocity, and individual impulse rate histograms from the 17 spindle afferents studied. The vertical line marks the transition between lengthening and shortening phases of the movement. The bars to the right of the diagrams indicate a rate of 8 impulses/s Asterisks indicate cycles which were free from EMG activity of the parent muscle (*see* text). To the left of the individual rate histogram, unit code number and unit classification code are given (*see* text) Movement amplitude was 25° and peak velocity 20 deg/s. Note that position and velocity scales do not cover complete ranges

lengthening and low or absent during shortening. In contrast, a static response dominated completely in 2 units (13, 14) which produced essentially the same rate during lengthening and shortening. On the other hand, it was obvious that a fair proportion of units exhibited intermediate characteristics in this respect. All the spindle afferents which originally were classifed as primaries (n = 8) or secondaries (n = 1) exhibited the expected response profile, namely, a high or a low dynamic stretch response to imposed pseudosinusoidal movements. They are indicated by Ia and II without brackets in fig. 2. As was pointed out in the Methods section, the classification data were ambiguous with 8 spindle afferents with regard to the unit being a primary or a secondary afferent. However, 5 of these units exhibited a distinctly high or low dynamic response to the imposed movements as illustrated in fig. 2, where they are indicated by Ia and II symbols within brackets. On the basis of these findings, it seemed reasonable to classify them as primaries or secondaries. Thus the spindle sample probably consisted of 12 primaries and 2 secondaries, 3 units not being classifed in this respect. Fig. 2 suggests a continuity between units having a high dynamic response and those having a low dynamic response to the imposed movement rather than two groups with distinctly different properties.

Contractions during imposed movements. Several spindle afferents exhibited obvious deviations from a regular stretch response to imposed movements (fig. 2). Three units which fired mainly during lengthening reached relatively high rates in the first half of the stretch phase and then, for about half a second, fell to considerably lower rates (spindle afferents 15, 16, 17). Others presented an accelerated rate in the later part of the shortening phase which is not easily interpretable as a pure stretch response (e.g., spindle afferent 12).

Further analysis indicated that the deviating features were often associated with involuntary contractions as illustrated by the 2 sample units in fig. 3. The unit in fig. 3A exhibited a seemingly clean dynamic response during the stretch phase of the two imposed movements. However, during shortening, the subject made an involuntary contraction which is evident in the torque record at the top. The extrafusal activation was associated with a relatively strong spindle firing. In fig. 3B two cycles from another unit are illustrated, one imposed and one active. The first train of impulses during the imposed movement appeared while the subject was contracting the parent muscle and it started exactly when the movement shifted from shortening to lengthening, suggesting a combination of stretch and fusimotor effects. The firing stopped when the EMG activity vanished in spite of the progressive stretch. After a pause, a second train of impulses of lower rate appeared when the muscle was close to its maximal length, suggesting a pure stretch response For comparison, the response during an active movement of similar velocity and amplitude is shown to the right in fig. 3B. The findings confirm Burke *et al.* (1987*b*) in demonstrating that small contractions during imposed movements may totally upset the regular stretch response of spindle afferents. It is tempting to speculate that the two states of the muscle spindle are matched by two different modes of information processing of the afferent discharge in the central nervous system.

Fig. 3 is also representative in illustrating that the involuntary contractions usually occurred with a short rather than a long muscle. Hence the contraction probably represents the Westphal phenomenon (Westphal, 1880; Angel, 1983).

In fig. 2, asterisks indicate totally clean cycles, that is, movements without contractions. They thus represent spindle responses from truly passive muscles. Nonmarked diagrams in fig. 2 were collected in runs when some activity was seen in the EMG records

Voluntary movements

The subjects were asked to perform a series of finger movements, roughly mimicking the preceding imposed pseudosinusoidal movements with regard to speed and amplitude.

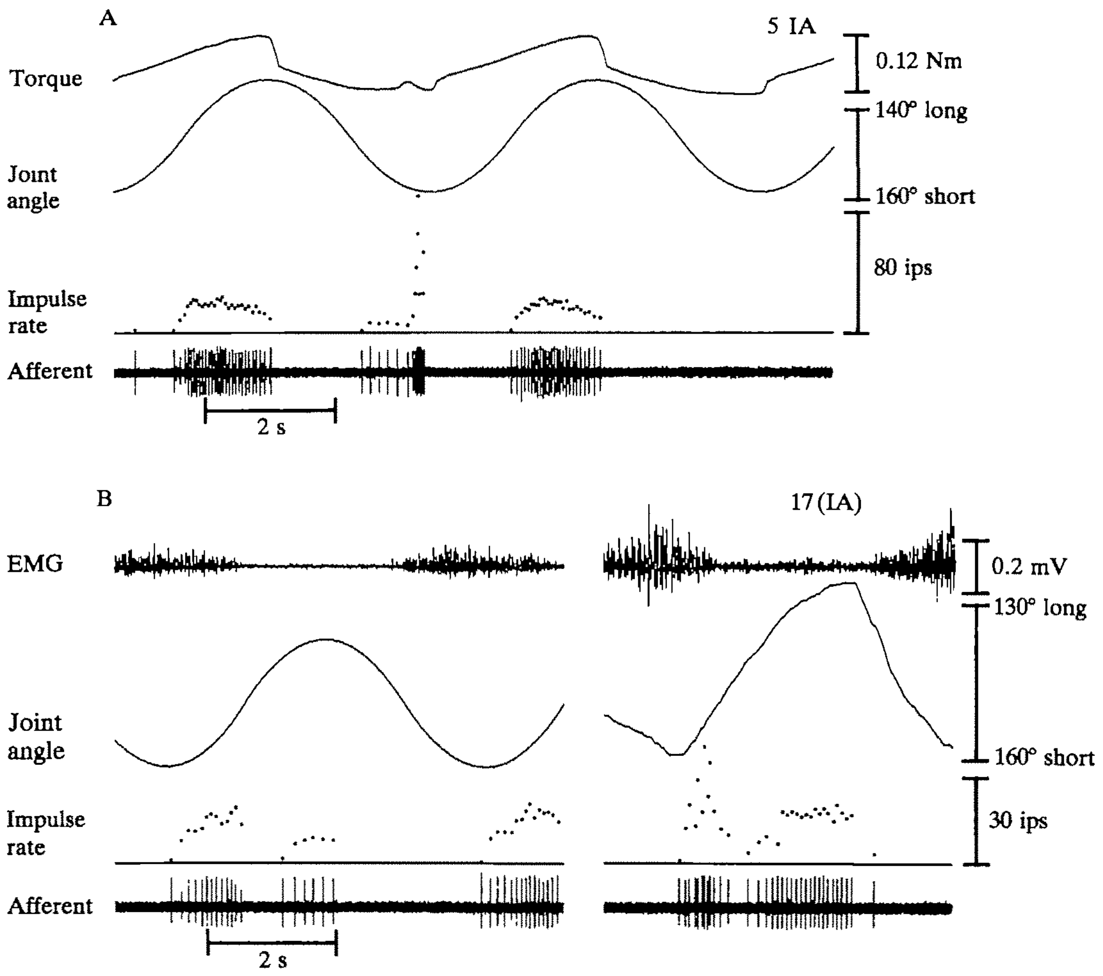

FIG. 3. Effects of contractions on spindle response to imposed movements and active movements with no load. A, two cycles of imposed pseudosinusoidal movements. The records show from the top, torque and angle at the metacarpophalangeal joint of the test finger, instantaneous impulse rate of a spindle primary afferent from the extensor muscle, and the raw microelectrode recording. B, imposed and active movements with another muscle spindle primary afferent. Similar traces as in A except that top trace shows unprocessed EMG activity. Unit code numbers and classification symbols are shown to the right above the two diagrams (*see* Table 1). (ips = impulses per second.)

Fig. 4 shows individual and representative sample runs from all spindle afferents with movements opposed by a small constant torque load (5 − 15% of maximal voluntary torque). Amplitudes of active movements were relatively uniform and similar to the imposed movements with all subjects, i.e., about 25°. However, the speed varied considerably between subjects as evident from the durations of the cycles.

A comparison between figs 2 and 4 indicates that the discharge profiles of many units were broadly similar with imposed and active movements. In both tests, the rate was generally higher during lengthening than during shortening, suggesting that spindle afferents provided stretch responses with passive as well as active movements. Moreover, the static/dynamic relation was roughly similar in many units, i.e., units with a preferentially dynamic response to imposed movements exhibited also a more pronounced dynamic response to active movements and vice versa. On the other hand, there were also systematic differences between active and imposed movements. Most obviously, the interspike interval variability was considerably higher during active movements with many of the primaries, whereas the 2 secondaries exhibited a low variability during either type of movement. The mean impulse rate as well as the peak rates were only moderately higher during active movements in some units whereas it was similar in many of them.

Although most of the spindle afferents provided movement monitor profiles during active movements, a few of them exhibited different profiles (fig. 4). One primary afferent (unit 5) produced a flat response without any clear modulation related to stretch. It is difficult to decide whether it was relevant that this subject performed slightly slower movements than any of the others. Unfortunately, only 1 unit was studied in this subject. One (unit 10) produced a much larger discharge during active shortening than during lengthening, as will be further elaborated below. Finally, 2 units (1, 9) produced appreciable discharges late during shortening which were not present with imposed movements.

Spindle response profiles related to size of load. The responses shown in fig. 4 originate from voluntary movements with small opposing loads but they are, with regard to the general response profiles, also representative of unopposed movements in most cases. Although the effect of load on impulse rate varied to some extent between units, the general response profile of the individual unit was usually similar with varying loads. It should be emphasized, however, that only loads of moderate size were tested, up to 15% of maximal voluntary torque.

More complete records illustrating the varying effects of load are shown in fig. 5. Sample runs from a unit with negligible effects are displayed in fig. 5A. It is noteworthy that the EMG, in contrast to the spindle rate, was considerably larger in the loaded movement, whereas spindle rate was similar. The lack of an effect of load, as in fig. 5A, was found in 47% of the spindle afferents (8 out of 17). However, in another 47% the impulse rate increased slightly when a small load was added, as illustrated by the sample records in fig. 5B. Moreover, 1 unit had an exceptional load response (fig. 5C). The imposed movement as well as the unloaded active movements were associated with regular stretch responses. When a small load was added, however, the rate increased

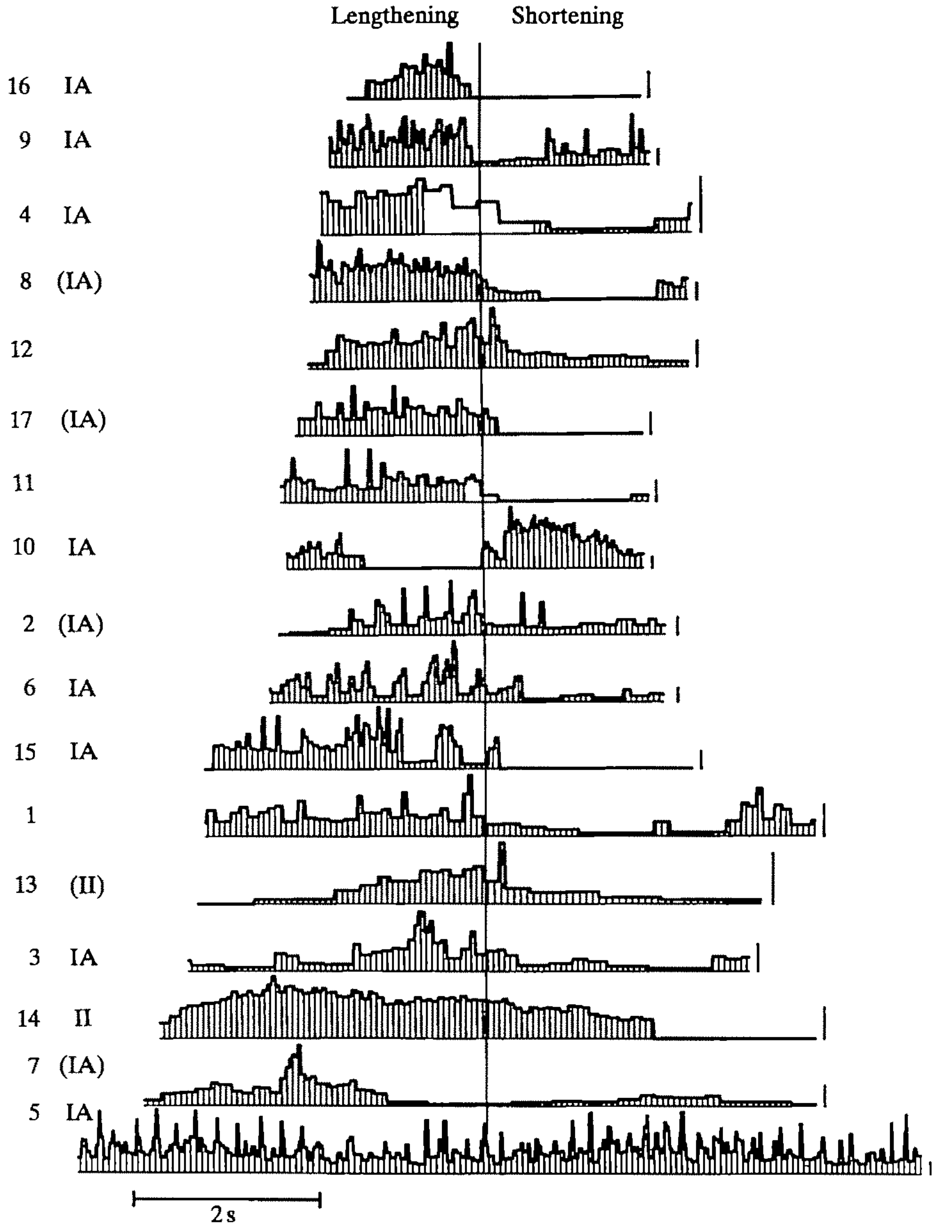

FIG. 4. Spindle response to active movements with small constant torque load opposing the extensor muscle. Individual sample runs from all the studied spindle afferents. The subjects were asked to mimic imposed movements illustrated in fig. 2. The vertical line marks the transition from lengthening to shortening phases. The white areas in the middle of two diagrams represent a phase when the subjects were holding a stretch position which some of them did in spite of explicit instructions to produce continuously alternating movements. Bars to the right indicate 16 impulses/s. Unit code numbers and classification symbols to the left of the histograms as in fig. 2.

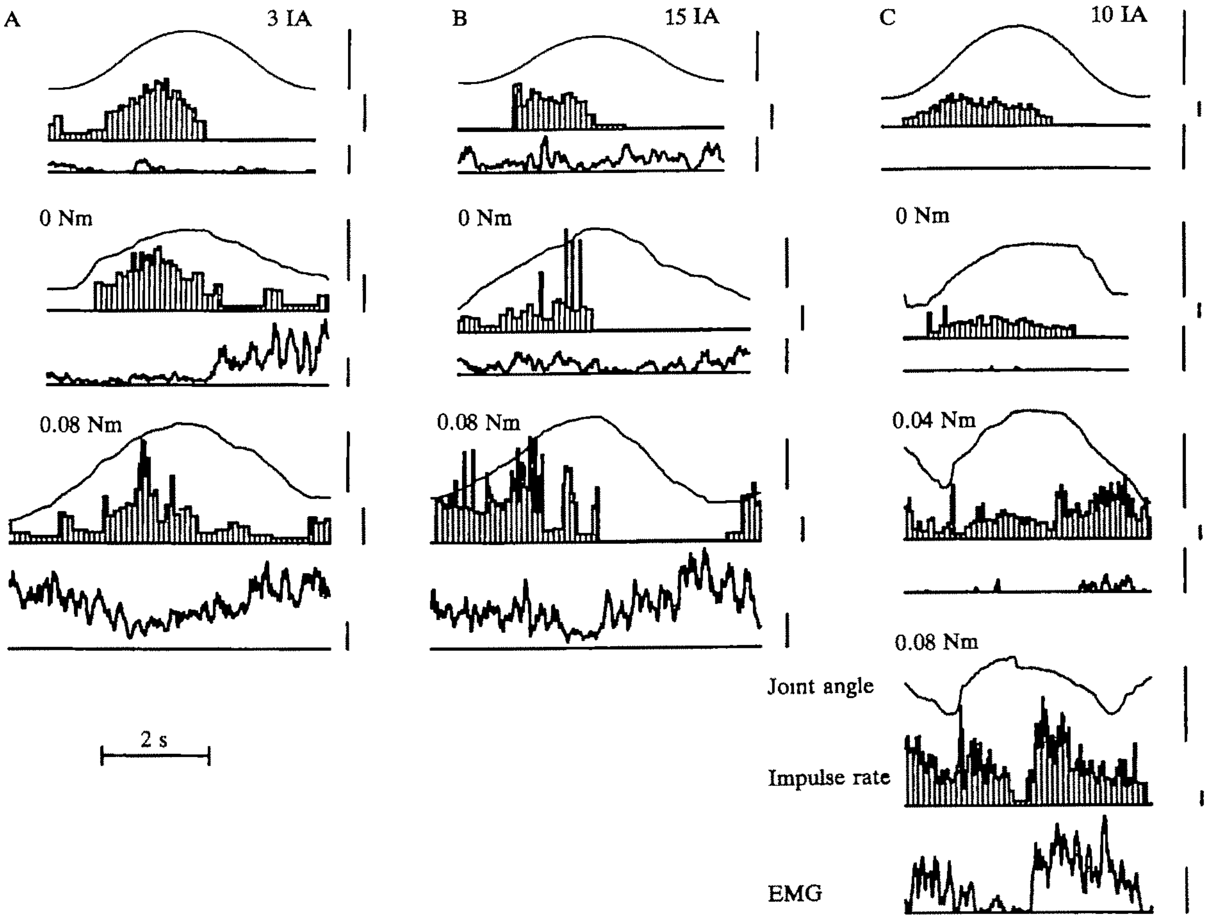

FIG 5. Load effect on spindle response to active movements Data from 3 different spindles are shown in A, B and C with an imposed movement at the top followed by active movements with different loads. The numbers to the left of the diagrams indicate the size of the opposing load. Unit code number and classification are given to the right. Traces show, from above, metacarpophalangeal joint angle of the test finger, impulse rate of the spindle afferent, and rectified and low-pass filtered EMG (4 Hz). The calibration bars indicate, from above, joint angle, 25°, impulse rate, 10 impulses/s, EMG activity, 0 1 mV.

considerably during shortening whereas the effect of load was minimal during lengthening (fig. 5c, third from top). As a result there was a shift from a regular stretch response profile with an unloaded muscle to a converse stretch response profile with a loaded muscle, that is, higher rates during shortening than during lengthening. A further increase of the load provided an impulse rate which was closely related to the envelope of the EMG activity (fig. 5G, bottom panel). It should be emphasized that the identification of this unit as a muscle spindle primary afferent was based on solid evidence (Table 1), including a positive stretch sensitization test and locking to small sinusoidal stretchings (*see* Methods).

Isometric versus isotonic contractions. Burke *et al.* (1978*c*) argued that, during isometric contractions, individual spindle afferents have different and invariant thresholds in terms of active force (i.e., rate acceleration does not occur until a characteristic force level of the parent muscle has been attained). In relation to this finding, it was of interest to compare the response of individual spindle afferents during isometric and isotonic contractions. An analysis of contingencies in the present sample revealed that most of the units which accelerated with weak isometric contractions (7 out of 9), also exhibited a higher impulse rate during active movements with no extra load as compared with the rates during imposed movements, indicating an increased fusimotor drive in both types of contraction. On the other hand, all the spindle afferents which failed to accelerate with isometric contractions (8 units) provided approximately similar rates during passive imposed and voluntary movements, suggesting a poor fusimotor drive in isometric as well as isotonic contractions.

Golgi tendon organs

The group of tendon organ afferents (n = 8) exhibited altogether different response patterns than the spindle afferents (fig. 6). First, their discharge was not modulated by imposed stretch when the muscle was passive (fig. 6, left panel). Two units were not tested with imposed pseudosinusoidal movements but their lack of stretch sensitivity was documented using ramp movements. One single unit provided a converse stretch response (unit 6), probably as a result of involuntary muscular contraction during the imposed movements (*see below*). Thus none of the Golgi tendon organ afferents produced a stretch response to imposed movements as tested in the present study, in contrast to the spindle afferents, nearly all of which did (94%).

With active movements, the tendon organ afferents often produced a converse stretch response with minimal rates at maximal muscle length and often maximal rates during shortening (fig. 6, right panel). The impulse rate usually bore a positive relationship to the amount of EMG, as illustrated for a sample unit in fig. 7. These findings clearly support the view that Golgi tendon organs monitor muscle contraction during active movement.

Comparison between spindle and Golgi afferents

Afferent impulse rate versus position, velocity, and EMG

Although the diagrams of figs 4–7 demonstrate, in general, stretch responses from spindle afferents and contraction responses from tendon organ afferents during active movements, it seemed of interest to quantify these responses. Linear regression analysis

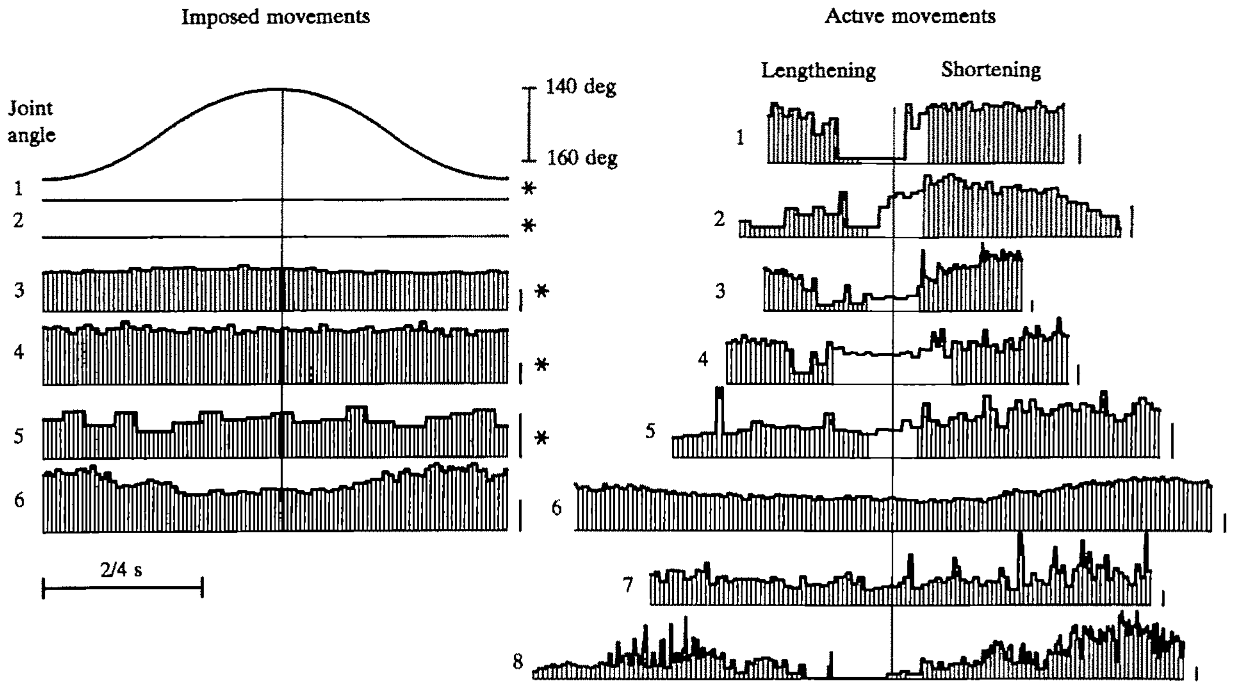

FIG. 6. Response of all Golgi tendon organs studied to imposed and active movements with a small constant torque load opposing the extensor muscle. Individual single runs from all the tendon organ afferents studied are shown in the *right panel* whereas data from 2 units are lacking with imposed movements (*left panel*). Same layout as in figs 2 and 4 Asterisks in the left panel indicate cycles without involuntary contractions of the parent muscle. Calibration bars to the right indicate 8 impulses/s. The time bar indicates 4 s for unit number 8 and 2 s for the others. Unit code numbers are given to the left (*see* Table 1 for details).

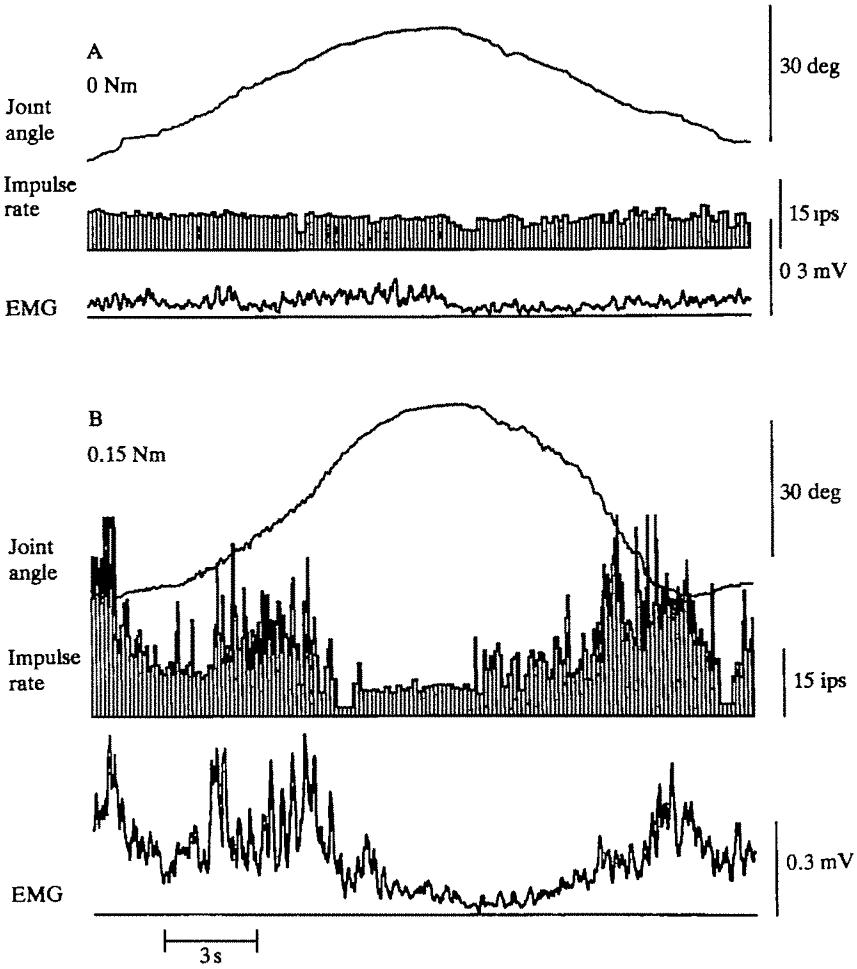

FIG. 7. Response of Golgi tendon organ afferent (unit 7) to active movements. Traces represent, from above, metacarpophalangeal joint angle of the test finger, impulse rate of the afferent and, at the bottom, rectified and low-pass filtered EMG (4 Hz). Numbers to the left indicate the size of the opposing load. (ips = impulses per second.)

was pursued between, on the one hand, instantaneous impulse rate and, on the other, movement velocity, joint position, and EMG activity of the parent muscle during the active movements. The analysis was based on data strings of 15–25 s duration from the individual unit but it was limited to those afferents which were satisfactorily classified on the basis of the original identification tests.

There was a considerable scatter in the individual plots but a significant correlation was present with a fair proportion of the units. Table 2 shows that most spindle afferents, primaries as well as the nondifferentiated spindle afferents, exhibited a significant correlation between instantaneous impulse rate and movement parameters, whereas most tendon organ afferents exhibited a significant correlation between rate and amount of

TABLE 2 PERCENTAGE OF UNITS EXHIBITING SIGNIFICANT POSITIVE CORRELATIONS BETWEEN IMPULSE RATE AND VELOCITY, POSITION, AND EMG DURING ACTIVE MOVEMENTS

	No	*Velocity*	*Position*	*EMG*
IA	8	75	50	25
MS	8	75	38	0
IB	4	0	0	75

The entries in the first column of the table give the number of the studied afferents whereas the other three columns present the percentage of units of three categories exhibiting significant correlations ($P < 0.005$) in linear regression analysis between instantaneous impulse rate on the one hand, and velocity, position, and EMG activity on the other, during active movements performed with a small opposing load. The type of movements analysed is illustrated in fig. 1B. MS = muscle spindle afferents which were not classified as primaries or secondaries.

EMG activity. The correlation coefficient for the units represented in Table 2 was 0.5 ± 0.21 (mean ± SD).

The data in Table 2 support the interpretation that Golgi tendon organs provide information about the amount of muscle recruitment rather than stretch also during active movements and that muscle spindles provide stretch response in spite of the fusimotor drive associated with the contractions. Moreover, these findings indicate that active movements of this kind may be of diagnostic value for unit classification.

Spontaneous discharge of muscle afferents

With relaxed muscles, a fairly large proportion, 63% (5 out of 8) of Golgi tendon organs were continuously discharging against not more than 35% (6 out of 17) of the spindle afferents. The high proportion of spontaneously discharging Golgi tendon organs is probably not representative but may simply be an effect of selection bias related to the search procedure (*see* Methods).

DISCUSSION

Response profiles of muscle spindle afferents

Three different response profiles were found among spindle afferents during active routine movements with small loads. Purely descriptive terms were used to denote the three profiles—stretch response profile, flat profile, and converse stretch response profile. This categorization was preferred to statements that spindle afferents are dominated either by length changes or by fusimotor activity (Prochazka *et al.*, 1979), partly because the flat response profile is difficult to accommodate in these two categories.

Stretch response profile. Mere inspection of the cycle histograms as well as statistical analyses indicated that most spindle afferents produced a stretch response during active movements. The Ia afferents usually provided a continuous discharge during lengthening whereas they were largely silent during shortening. This was true for unopposed movements as well as for movements opposed by small constant torque loads in most cases.

In relation to the autogenic reflex effects, this finding may at first sight seem puzzling. Ia firing occurred mainly during lengthening when the muscle was not contracting and motoneuron excitability was depressed, preventing the emergence of reflex effects (Al-Falahe and Vallbo, 1988). This suggests that autogenic effects from Ia afferents were lacking altogether in these movements. It should be emphasized, however, that very simple routine movements were studied and the subjects were handling a very simple load. In many natural movements, the conditions would be different for the simple reason that movements are the result of a balanced cocontraction of agonists and antagonists, as would be the case, for instance, with high precision movements, with handling of loads of unknown size and nature, or during motor learning (Smith, 1981).

The cocontraction implies that motoneuron excitability is high all through the movement cycle and contraction would therefore be modulated by the Ia afferents. Moreover, the contracting antagonist provides an extra load which must be overcome during shortening. The stronger contraction during this phase might promote spindle firing in face of the ongoing shortening movement, as previously reported with external loads (Burke *et al.*, 1978*b*) and also found in the present study (spindle afferent 10).

On the other hand, it is of interest to discuss pathways other than the direct connections onto the muscle's own motoneurons when the role of the spindle stretch response is considered. It is well known that the effect from primary spindle afferents is not limited to the parent muscle. An important effect is exerted on the antagonists through an Ia inhibitory interneuron. Evidence has recently been accumulating that the control effect from spindles in a noncontracting muscle, being passively stretched, on its antagonists may be as powerful as the autogenic effect (Matthews and Watson, 1981; Hasan and Sasaki, 1986). Moreover, it seems likely that the afferent discharge from the passively stretched muscle has a sensory role and contributes to position sense and kinaesthesia as well as subconscious sensorimotor functions, for example, detection of error between intended and actual movement, coordination of muscle activity, initiation of triggered reactions, and updating of the 'body image' (Goodwin *et al.*, 1972; Crago *et al.*, 1976; Capaday and Cooke, 1981; Roll and Vedel, 1982; Bullen and Brunt, 1986).

Aberrant spindle response patterns. For 2 units, the discharge during active movements lacked an overt stretch response. One of them produced a flat response, that is, an impulse rate that was largely unmodulated by the gross movements and lacked obvious relations to position or direction of movement. A similar flat profile has been described in human subjects, particularly with slower movements than those in the present study (Vallbo, 1973; Burke *et al.*, 1978*b*; Hulliger *et al.*, 1985). It remains to be clarified whether this type of response is accounted for by a low sensitivity to stretch associated with a high static fusimotor drive, or a fusimotor drive which continuously compensates for the length changes produced by the muscle, as predicted by the servo-assistance hypothesis (Matthews, 1964).

A converse stretch response was seen with only one single unit. This type of response has previously been found in several preparations and mainly with slow movements. Critchlow and von Euler (1963) found that it is dominating among spindle afferents from the intercostal muscles in the cat during respiratory movements. Later, it was described in various subhuman preparations (Severin *et al.*, 1967; Larson *et al.*, 1983; Loeb, 1984) as well as human subjects (Burke *et al.*, 1978*b*; Vallbo, 1981; Hulliger *et al.*, 1985).

Since the converse stretch response usually implies higher impulse rates with stronger contractions of the parent muscle, the afferent rate is positively related to the envelope of the integrated EMG (Burke *et al.*, 1978*b*), as reported with tendon organ afferents. Thus there is good reason to ask whether human spindle afferents which produce the converse stretch response are, in fact, misclassified tendon organ afferents.

It is beyond doubt that the critical unit of the present study was a spindle primary afferent since it exhibited a large number of characteristic features (Table 1A, unit 10), including stretch sensitization and driving with low amplitude sinusoidal stretch. Burke *et al.* (1978*b*) reported that their spindle afferents whose rate followed 'the integrated EMG envelope rather than the length change' usually differed from the main group of spindle afferents in two respects. They were influenced only slightly by passive movements of the ankle joint when the muscles were relaxed. Moreover, 'each responded vigorously during isometric voluntary contractions'. It is interesting that these two features are characteristic of all our tendon organ afferents. Since the classification of Burke *et al.* (1978*b*) was not supported by identification tests other than the twitch test, it seems difficult to judge whether all their spindle afferents were correctly classified (Edin and Vallbo, 1987). On the other hand, a survey of the literature (*see* above) strongly suggests that the converse stretch profile is a genuine response of spindle afferents in animals as well as in conscious human subjects. A feasible interpretation is that beta-activation contributes to the converse stretch response (Grill and Rymer, 1987).

The clarification of the significance of the varying response profiles of muscle spindles constitutes a major problem, particularly since the responses may be mutually opposite in monitoring the major physical input to the sense organ, that is, length and velocity.

Determinants of muscle spindle response profiles

Isometric versus isotonic contractions. A stretch response profile has been emphasized as the regular response of spindles in behaving animals (Loeb and Duysens, 1979; Prochazka *et al.*, 1979; Larson *et al.*, 1983; Loeb *et al.*, 1985). In contrast, a crude parallelism between spindle afferent rate, on the one hand, and force and/or EMG activity on the other, is common with isometric contractions in human subjects (Vallbo, 1974; Burg *et al.*, 1976; Burke *et al.*, 1978*a*; Vallbo and Hulliger, 1982). These findings may at first sight be interpreted as indicating basically different fusimotor drives. However, the present findings eliminate this seeming discrepancy because they demonstrate that many spindles in human subjects respond qualitatively in the same way as spindles in behaving animals during simple isotonic movements of moderate speed.

One reason for the seeming discrepancy between isometric and isotonic contractions might simply be that a dependence of spindle firing on degree of muscle recruitment is more evident in the former type of contraction. A mere inspection of a single isometric contraction reveals whether spindle rate is positively related to EMG or not. On the other hand, to demonstrate a dependence on the degree of muscle recruitment during active movement requires a comparison between two isotonic contractions with similar movement parameters but different recruitment of the parent muscle. This test is usually not pursued in studies which emphasize the lack of parallelism between spindle rate and degree of muscle recruitment. In addition, a sampling bias may also be involved. The finding of a parallelism between impulse rate and isometric force in humans was, of course, based on studies of those spindle afferents which exhibited a rate acceleration during isometric contractions. On the other hand, studies of isotonic contraction usually also include units which lack a substantial fusimotor drive during weak contractions.

Size of load. The present findings support the view that the size of the opposing load is a factor of significance not only for the general level of discharge from spindle afferents but sometimes for the response profile as well. A load dependence of the response profile, as found in the present study, has been demonstrated in two previous studies in man with a shift from a clear stretch response with a small load to a less pronounced stretch response or even a converse stretch profile when the load increased (Burke *et al.*, 1978*b*; Hulliger *et al.*, 1985). It remains to be assessed whether a converse stretch profile is a regular response of all spindle afferents when sufficiently large loads are opposing the movement, or whether it occurs exclusively in a particular group of primary afferents. Moreover, it remains to be demonstrated whether the determinant factor is actually the size of the external load per se or the degree of recruitment of the working muscle.

Movement velocity. It has been inferred that the discharge pattern of spindle afferents is highly dependent on the speed of movement which would explain a difference between the dominating findings in man and behaving animals (Prochazka *et al.*, 1979; Loeb, 1984). A comparison of the present findings with previous results from the same muscles studied with much slower movements (Hulliger *et al.*, 1985) is clearly consistent with the general idea that higher speeds of movement favour stretch response profiles. On the other hand, the present study clearly confirms previous findings in man that different response profiles may occur with the same speed of movement (Hulliger *et al.*, 1985), suggesting that a particular range of joint velocities does not provide a uniform response profile from muscle spindles during active movement.

Comparison between muscle spindle and tendon organ discharge

The two groups of units classified as muscle spindles and Golgi tendon organs exhibited clearly different response profiles during imposed as well as active movements of intermediate speed. With a few exceptions, the spindle afferents responded to stretch in both types of movement. In contrast, all the tendon organ afferents were unmodulated by imposed stretch in the relaxed muscle. During active movements, many of them fired in fair relationship to the envelope of the rectified EMG. As a result they often provided a converse stretch response with maximal rates in a shortened muscle and minimal rates in a lengthened muscle, consistent with previous findings in man (Burke *et al.*, 1978*b*) and cats (Prochazka and Wand, 1980; Appenteng and Prochazka, 1984). Thus there was no indication that the Golgi tendon organs monitored muscle length in movements opposed by small constant torque loads, in contrast to the suggestion by Larson *et al.* (1983). Apparently the stretch effect of the voluntary movement (Houk and Henneman, 1967; Appenteng and Prochazka, 1984) was over-compensated by the effect of extrafusal contraction.

To summarize, the present findings indicate that, during routine movements of moderate speed which were unopposed or opposed by only small loads, human tendon organs behave as monitors of the amount of muscle contraction whereas muscle spindles behave as stretch receptors. However, a few puzzling exceptions were found which could not be discarded as misclassified units.

ACKNOWLEDGEMENTS

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HEREDITARY MOTOR SYSTEM DISEASES (CHRONIC JUVENILE AMYOTROPHIC LATERAL SCLEROSIS)

CONDITIONS COMBINING A BILATERAL PYRAMIDAL SYNDROME WITH LIMB AND BULBAR AMYOTROPHY

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SUMMARY

Forty-three patients with hereditary motor system diseases belonging to 17 families were studied. The clinical features consisted of a bilateral pyramidal syndrome, weakness with atrophy and fasciculation of the hands and/or the legs, with or without a bulbar or a pseudobulbar syndrome and without sensory disturbance. Electromyography in 31 cases (including all index cases) showed evidence of denervation. Motor and sensory nerve conduction velocity was normal; sensory nerve action potential amplitudes, examined in 11 cases, were also normal. Nerve and muscle biopsies taken in 29 cases (including all index cases) showed neurogenic atrophy in the peroneus brevis muscle and minor changes only in the superficial peroneal nerve. The mean age of onset was 12.06 (range 3–25 years), and progression was very slow. Inheritance appeared to be autosomal recessive. Depending on the clinical presentation, the patients were subdivided into three groups comprising (1) upper limb and sometimes bulbar amyotrophy with a bilateral pyramidal syndrome (17 patients: 11 familial and 6 isolated); (2) spastic paraplegia with peroneal muscular atrophy (14 patients: 11 familial and 3 isolated); and (3) a spastic pseudobulbar form (12 patients in a large kinship). These entities are discussed and compared with other cases reported in the literature.

INTRODUCTION

The association of a bilateral pyramidal syndrome with amyotrophy in the limbs and fasciculation in a young subject (infant or adolescent) raises an important nosological distinction between classical amyotrophic lateral sclerosis (ALS), or 'Charcot's disease', and more protracted and often inherited forms. The term juvenile ALS has sometimes been used to designate such syndromes, even in the absence of anatomical documentation (van Bogaert, 1925; Ford, 1960; Bonduelle, 1975, 1982; Emery and Holloway, 1982; Ben Hamida and Hentati, 1984). With the exception of rare instances of rapidly progressive juvenile ALS, comparable with Charcot's disease (Berry *et al.*, 1969; Nishigaki *et al.*, 1971; Nelson and Prensky, 1972), this is usually a chronic disease. It is sometimes isolated but quite often familial, and can take various forms. In its commoner form, it variously combines amyotrophy and fasciculation of the hands with spastic paraplegia and a bulbar or pseudobulbar syndrome. We have grouped with

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this commoner form other familial or isolated cases with a juvenile or infantile onset in which a spastic paraplegia is associated with distal amyotrophy in the lower limbs, with or without a spastic pseudobulbar syndrome.

PATIENT AND METHODS

The patients were examined at or investigated in hospital at the National Institute of Neurology between 1974 and 1988. Cases of severe spinal amyotrophy of the Werdnig-Hoffman type (death within 2 yrs of birth), the infantile and juvenile forms of progressive proximal spinal muscular atrophy (SMA), and the scapuloperoneal atrophies, were excluded. We also excluded 5 cases conforming to the definition of the Vialetto-Van Laere syndrome (progressive bulbar paralysis with deafness (Vialetto, 1936; Van Laere, 1966)) which probably constitutes a separate genetic entity.

The criteria for diagnosis were (1) onset usually before the age of 25 yrs; (2) the simultaneous or dissociated appearance of muscle weakness with atrophy and fasciculation in the arms and sometimes in the legs, a pyramidal syndrome, and labioglossolaryngeal paralysis and/or pseudobulbar syndrome; (3) a neurogenic electromyogram (EMG) with normal motor nerve conduction velocity (MNCV); (4) normal sensory nerve conduction; and (5) denervation changes on muscle biopsy and normal appearance on sensory nerve biopsy.

Of the 17 cases chosen, a survey in the field made it possible to reexamine all the living index cases, to determine their current condition, to examine all available family members, and to discover 26 secondary cases belonging to 8 families. The group studied thus comprised 43 patients belonging to 17 families. A family pedigree was established in all the familial cases. In consanguineous kindreds the coefficient of inbreeding (CI) was calculated by the method of Wright (1922). Segregation analysis was performed using the sibship method (Fisher, 1934) and the Weinberg proband method (Weinberg, 1928).

Superficial peroneal nerve and peroneus brevis muscle biopsies were performed in 29 cases, including all the index cases. The sections were studied using previously described techniques (Ben Hamida *et al.*, 1983, 1987). The cerebrospinal fluid (CSF) protein content was measured in 34 cases. The results were analysed statistically using Student's t test, and the results expressed as means ±2 SEM.

We identified three different clinical presentations: (1) upper limb (±bulbar) amyotrophy with bilateral pyramidal involvement; (2) spastic paraplegia with peroneal muscular atrophy; and (3) a spastic pseudobulbar syndrome with spastic paraplegia.

CASE REPORTS

Group 1: upper limb (±bulbar) amyotrophy with bilateral pyramidal involvement

This group was characterized by muscle atrophy and a pyramidal syndrome with or without bulbar involvement. The muscle atrophy began in the small hand muscles (fig. 1), sometimes extending progressively in the upper limbs to the shoulders. Fasciculation was visible in the atrophic muscles. The pyramidal syndrome produced a spastic gait. The bulbar syndrome appeared gradually in the form of labioglossopharyngeal paralysis. There was no bladder dysfunction and no sensory disturbance. In this group there were 17 cases belonging to 10 families; 11 were familial and 6 were isolated. The clinical features of the index cases are detailed below and those of affected members are summarized.

Family 1

Case 1, propositus, 28-yr-old male. Onset was at 10 yrs with weakness. Examination showed a spastic gait, brisk tendon reflexes, extensor plantar responses, atrophy of the hand muscles with fasciculation, and no bulbar signs. CSF protein: 0.20 g/l. Muscle biopsy: denervation; nerve biopsy: normal. EMG: denervation in all four limbs. MNCV: 51 $m \cdot s^{-1}$ (ulnar), 48 $m \cdot s^{-1}$ (peroneal). Sensory nerve action potential (SAP) amplitude: 28 μV (median), 11 μV (sural); sensory nerve conduction velocity (SNCV): 50 $m \cdot s^{-1}$ (sural), 50 $m \cdot s^{-1}$ (median).

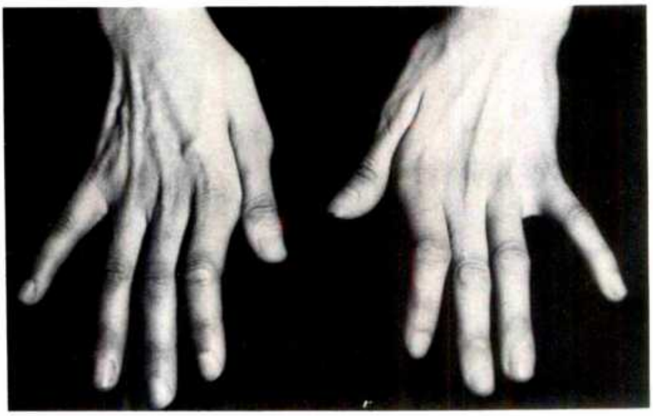

FIG. 1. Atrophy of small hand muscles in a 17-yr-old girl (*Case 12*).

Case 2, the 36-yr-old bedridden sister of Case 1 whose history was identical to that of the propositus. CSF protein: 0.15 g/l.

Family 2

Case 3, propositus, the 18-yr-old daughter of consanguineous parents (CI = 1/16). Onset was at 16 yrs with progressive difficulty in handling articles and in walking. Examination showed a spastic gait, extensor plantar responses, moderate atrophy of the muscles of the left hand with fasciculation, and no bulbar signs. CSF protein: 0.27 g/l. Muscle biopsy: denervation; nerve biopsy: normal. EMG: denervation in all four limbs. MNCV: 40 $m \cdot s^{-1}$ (median), 58 $m \cdot s^{-1}$ (ulnar), 44 $m \cdot s^{-1}$ (peroneal). SAP amplitude: 18 μV (ulnar), 18 μV (saphenous). SNCV: 60 $m \cdot s^{-1}$ (median), 58 $m \cdot s^{-1}$ (ulnar), 47 $m \cdot s^{-1}$ (peroneal).

Case 4, the 27-yr-old sister of Case 3. Onset was at 10 yrs with an identical clinical picture but, in addition, difficulty in swallowing and dysarthria. CSF protein: 0.57 g/l. Muscle biopsy: denervation; nerve biopsy: normal. EMG: denervation in all four limbs. MNCV: 65 $m \cdot s^{-1}$ (median), 45 $m \cdot s^{-1}$ (peroneal). SAP amplitude: 39 μV (median), 23 μV (saphenous). SNCV: 58 $m \cdot s^{-1}$ (median), 54 $m \cdot s^{-1}$ (saphenous).

Family 3 (fig. 2)

Case 5, propositus, the 16-yr-old daughter of consanguineous parents (CI = 1/16). From the age of 7 yrs progressive weakness of the left hand then of both hands developed. A few years later difficulty in walking appeared. Examination showed a spastic gait and marked amyotrophy of the hands. There were no bulbar signs. CSF protein: 0.11 g/l. Nerve biopsy normal; muscle biopsy: denervation. EMG: denervation in all four limbs. MNCV: 38 $m \cdot s^{-1}$ (ulnar), 35 $m \cdot s^{-1}$ (peroneal). SAP amplitude: 45 μV (median), 26 μV (ulnar), 26 μV (saphenous). SNCV: 45.5 $m \cdot s^{-1}$ (saphenous), 52 $m \cdot s^{-1}$ (ulnar), 56 $m \cdot s^{-1}$ (median).

Case 6, the 12-yr-old sister of Case 5. Onset was at 6 yrs. The course was more aggressive than in the propositus.

Family 4

Case 7, propositus, 30-yr-old female. At the age of 16 yrs progressive difficulty in walking appeared. On examination, her gait was spastic and waddling. There were pyramidal signs in all four limbs with spasticity, brisk tendon reflexes apart from absent ankle jerks, extensor plantar responses and amyotrophy of the hands with fasciculation. EMG: denervation. MNCV: 39 $m \cdot s^{-1}$ (peroneal), 58 $m \cdot s^{-1}$ (ulnar). CSF protein: 0.21 g/l. Nerve biopsy: normal; muscle biopsy: denervation.

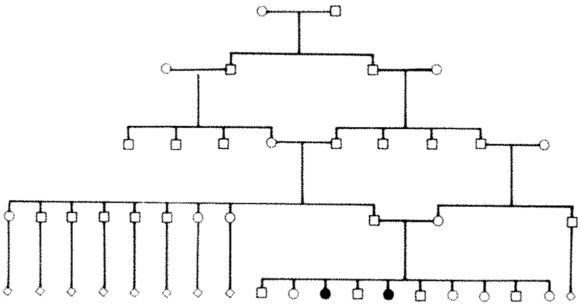

FIG. 2. Pedigree of family 3. □ = male nonaffected; ■ = male affected; ○ = female nonaffected; ● = female affected.

Case 8, the 33-yr-old brother of Case 7. Onset was at 13–14 yrs with an identical clinical picture.

Case 9, the 25-yr-old brother of Case 7 with an identical history.

Case 10, the 23-yr-old sister of Case 7 with an identical history. CSF protein: 0.20 g/l. Nerve biopsy: normal.

Case 11, the 13-yr-old sister of Case 7 with an identical history.

Family 5

Case 12, propositus, the 17-yr-old daughter of consanguineous parents (CI = 1/16). At the age of 13 yrs, the weakness of the right arm, then of the left arm, and then of the lower limbs appeared. Examination showed wasting of the hands, a spastic paraplegia, brisk tendon reflexes, bilateral extensor plantar responses and pes cavus. Bulbar involvement (mild atrophy of tongue and dysarthria) developed recently. EMG: denervation. MNCV: 40 $\text{m} \cdot \text{s}^{-1}$ (peroneal), 51 $\text{m} \cdot \text{s}^{-1}$ (ulnar). CSF protein: 0.22 g/l. Nerve biopsy: normal; muscle biopsy: denervation. Hexosaminidase activity: A, 1268 U/l, B, 556 U/l (normal).

Family 6

Case 13, propositus, the 20-yr-old son of consanguineous parents (CI = 1/16). Onset was at 6 yrs. Examination showed muscle wasting in the hands with fasciculation, and brisk tendon reflexes in all four limbs. EMG: denervation. MNCV: 40 $\text{m} \cdot \text{s}^{-1}$ (peroneal), 50 $\text{m} \cdot \text{s}^{-1}$ (ulnar). CSF protein: 0.17 g/l. Muscle biopsy: denervation; nerve biopsy: normal.

Family 7

Case 14, propositus, the 19-yr-old daughter of consanguineous parents (CI = 1/16). Onset was at 12 yrs. Examination showed distal amyotrophy in the upper limbs with fasciculation, a spastic paraparesis, brisk tendon reflexes, extensor plantar responses, and a bulbar syndrome with palatal paralysis, marked atrophy of the tongue and difficulty in swallowing. EMG: denervation. MNCV: 41 $\text{m} \cdot \text{s}^{-1}$ (peroneal), 45 $\text{m} \cdot \text{s}^{-1}$ (ulnar). CSF protein: 0.15 g/l. Muscle biopsy: denervation.

Family 8

Case 15, propositus, a 38-yr-old male, the son of consanguineous parents (CI = 1/16). Weakness and atrophy of the hands appeared by the age of 24 yrs. Examination showed distal amyotrophy in the upper limbs with fasciculation, and pyramidal signs in all four limbs with brisk tendon reflexes and extensor plantar responses. A bulbar syndrome developed at the age of 30 yrs with atrophy and fasciculation of the tongue. EMG: denervation. MNCV: 54 $\text{m} \cdot \text{s}^{-1}$ (ulnar), 41 $\text{m} \cdot \text{s}^{-1}$ (peroneal). CSF protein: 0.10 g/l. Muscle biopsy: denervation; nerve biopsy: normal.

Family 9

Case 16, propositus, a 30-yr-old male. At the age of 25 yrs, there was weakness and wasting of the hand muscles with fasciculation, pyramidal signs in all four limbs with brisk tendon reflexes and extensor plantar responses. Bulbar weakness appeared at the age of 28 yrs. EMG: denervation. MNCV: 53 $m \cdot s^{-1}$ (ulnar), 43 $m \cdot s^{-1}$ (peroneal). CSF protein: 0.23 g/l. Muscle biopsy: denervation; nerve biopsy: normal.

Family 10

Case 17, propositus, a 31-yr-old male, the son of consanguineous parents (CI = 1/16). At the age of 25 yrs there was weakness of the right hand. Examination showed distal amyotrophy, fasciculation, and a pyramidal syndrome in all four limbs, with brisk tendon reflexes and bilateral extensor plantar responses. MNCV: 55 $m \cdot s^{-1}$ (ulnar). CSF protein: 0.37 g/l. Muscle biopsy: denervation; nerve biopsy: normal.

Group 2: spastic paraplegia with peroneal atrophy

In this group there were 14 cases from 6 families (3 isolated cases and 11 familial cases with normal parents). This form was characterized by spastic paraplegia and lower limb atrophy (fig. 3) with frequent abolition of one or both ankle jerks. There was no bladder dysfunction and no sensory disturbance.

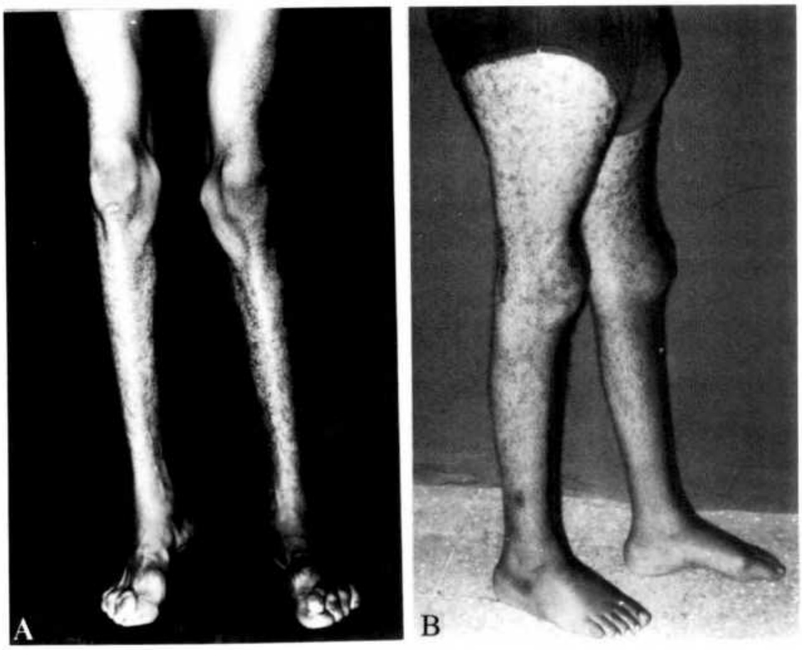

FIG. 3. A, marked atrophy of the legs (*Case 18*) and B, atrophy of lower part of thigh (*Case 25*).

Family 11 (fig. 4)

Case 18, propositus, a 27-yr-old male, the son of consanguineous parents (CI = 1/8). Onset was at 6–7 yrs, with progressive difficulty in walking. Examination showed a spastic gait, distal amyotrophy in the lower limbs, and brisk tendon reflexes. EMG: denervation. MNCV: 44 $m \cdot s^{-1}$ (peroneal). CSF protein: 0.23 g/l. Nerve biopsy: normal.

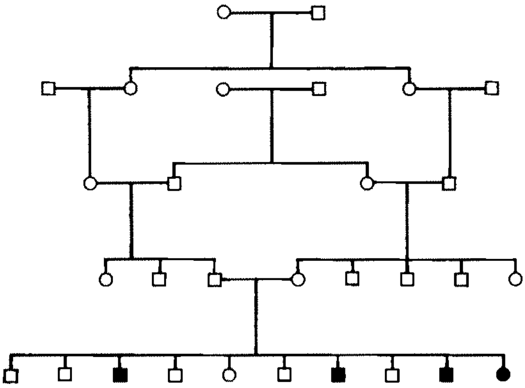

FIG. 4. Pedigree of family 11. Symbols as in fig. 2.

Case 19, the 31-yr-old brother of Case 18 with an identical clinical history. EMG: denervation. MNCV: 43 $m \cdot s^{-1}$ (peroneal). CSF protein: 0.16 g/l. Muscle biopsy: denervation; nerve biopsy: normal.

Case 20, the 16-yr-old sister of Case 18 with an identical history. EMG: denervation. MNCV: 52 $m \cdot s^{-1}$ (median), 51 $m \cdot s^{-1}$ (peroneal). CSF protein: 0.21 g/l.

Case 21, the 19-yr-old brother of Case 18 with an identical history. EMG: denervation. CSF protein: 0.20 g/l. Muscle biopsy: denervation; nerve biopsy: normal. Hexosaminidase activity: A, 1380 U/l, B = 950 U/l (normal).

Family 12

Case 22, propositus, a 16-yr-old male, the son of consanguineous parents (CI = 1/16). Onset was at 8 yrs with progressive difficulty in walking. Examination showed a spastic gait, severe peroneal atrophy with paralysis of the extensor muscles of the feet, brisk tendon reflexes, absent ankle jerks, and extensor plantar responses. EMG: denervation. MNCV: 38 $m \cdot s^{-1}$ (peroneal), 42 $m \cdot s^{-1}$ (ulnar). CSF protein: 0.13 g/l. Muscle biopsy: denervation; nerve biopsy: normal.

Case 23, the 20-yr-old brother of Case 22 with an identical history. EMG: denervation. MNCV: 41 $m \cdot s^{-1}$ (peroneal), 42 $m \cdot s^{-1}$ (ulnar). CSF protein: 0.18 g/l.

Case 24, the 14-yr-old brother of Case 22, again with an identical history. EMG: denervation. MNCV: 38 $m \cdot s^{-1}$ (ulnar), 31 $m \cdot s^{-1}$ (peroneal). The CSF protein was 0.12 g/l. Muscle biopsy: mild denervation; nerve biopsy: normal.

Family 13

Case 25, propositus, a 38-yr-old male, born to consanguineous parents (CI = 1/16). Onset was at 15 yrs with progressive difficulty in walking. Examination showed a bilateral steppage gait, peroneal atrophy, pes equinovarus, paralysis of the peroneal and triceps sural muscles, brisk tendon reflexes, and absent ankle jerks. CSF protein: 0.29 g/l. Muscle biopsy: denervation; nerve biopsy: normal. EMG: denervation in all four limbs. MNCV: 38.5 $m \cdot s^{-1}$ (peroneal), 52 $m \cdot s^{-1}$ (median), 50 $m \cdot s^{-1}$ (ulnar). SAP amplitude 24.5 μV (sural); SNCV: 40 $m \cdot s^{-1}$ (sural).

Case 26, a brother of Case 25 with an identical but less severe clinical picture. EMG: denervation in all four limbs. MNCV: 41 $m \cdot s^{-1}$ (peroneal), 48 $m \cdot s^{-1}$ (median), 47 $m \cdot s^{-1}$ (ulnar). SAP amplitude 11.5 μV (sural); 21 μV (median), 17 μV (ulnar). SNCV: 41 $m \cdot s^{-1}$ (sural), 58 $m \cdot s^{-1}$ (median), 46 $m \cdot s^{-1}$ (ulnar). CSF protein: 0 35 g/l.

Case 27, 25-yr-old male, a brother of Case 25 with an identical history. CSF protein: 0.17 g/l. Nerve biopsy: normal. EMG: denervation in all four limbs. SNCV: 41 $m \cdot s^{-1}$ (sural), 56 $m \cdot s^{-1}$ (median), 59 $m \cdot s^{-1}$ (ulnar); SAP amplitude: 13.5 μV (sural), 11.5 μV (ulnar). MNCV: 52 $m \cdot s^{-1}$ (median).

Case 28, 20-yr-old girl, the sister of Case 25 with an identical history. EMG: denervation. MNCV: 58 $m \cdot s^{-1}$ (median), 42 $m \cdot s^{-1}$ (peroneal); SAP amplitude: 14 μV (saphenous), 30 μV (median). SNCV: 45 $m \cdot s^{-1}$ (saphenous), 52 $m \cdot s^{-1}$ (median). Hexosaminidase activity: A, 1000 U/l; B, 300 U/l (normal).

Family 14

Case 29, propositus, 27-yr-old female, born to consanguineous parents (CI = 1/32). Onset was at 25 yrs, with difficulty in walking. Examination showed a steppage gait, paralysis of the extensors of the feet, amyotrophy of the hands, pyramidal signs in all four limbs with brisk tendon reflexes. The ankle jerks were absent. The plantar responses were not extensor. EMG: denervation. MNCV: 42 $m \cdot s^{-1}$ (ulnar). CSF protein: 0.22 g/l. Nerve biopsy: normal.

Family 15

Case 30, propositus, 29-yr-old male. Onset at 25 yrs with cramps and wasting of the left leg. Examination showed a steppage gait, muscle wasting in both legs, more severe on the left, fasciculation (thigh), brisk tendon reflexes, and an absent left ankle jerk. EMG: denervation. MNCV: 38 $m \cdot s^{-1}$ (peroneal), 45 $m \cdot s^{-1}$ (ulnar). Normal myelogram and lumbar CT scan. CSF protein: 0.22 g/l. Nerve biopsy: normal.

Family 16

Case 31, propositus, 28-yr-old male. Onset at 25 yrs with difficulty in walking. Examination showed a steppage gait, peroneal atrophy, fasciculation in the calves, brisk tendon reflexes and absent ankle jerks. EMG: denervation. MNCV: 38 $m \cdot s^{-1}$ (peroneal), 58 $m \cdot s^{-1}$ (median). CSF protein: 1.16 g/l. Normal nerve biopsy and myelogram.

Group 3: spastic pseudobulbar syndrome with spastic paraplegia

In this group all 12 cases belonged to one large family, apparently with autosomal recessive inheritance (fig. 5). This form presented an association of considerable spasticity of the facial muscles with uncontrolled laughter and weeping and a spastic dysarthria. Muscle atrophy and fasiculation were rare or absent and the gait was spastic. In some patients mild atrophy of the legs and/or of the hands was present (Fig. 6). There was no bladder dysfunction and there were no sensory disturbances.

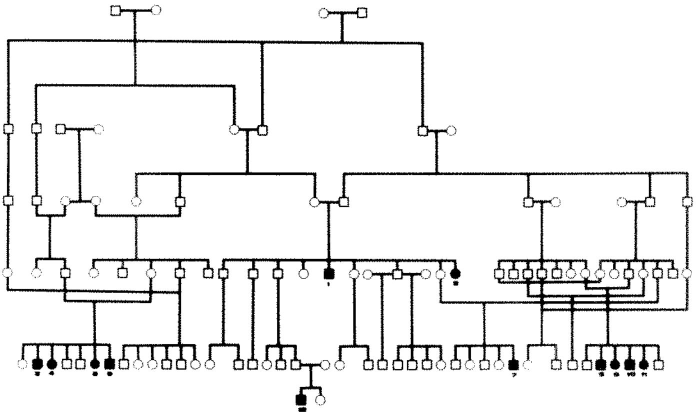

FIG. 5. Pedigree of the large family with spastic pseudobulbar syndrome and spastic paraplegia. Symbols as in fig. 2.

Family 17

Case 32, propositus, a 60-yr-old male, the son of consanguineous parents. Progressive dysphonia began at the age of 10 yrs and a progressive spastic paraplegia at age 20. He became bedridden at age 50. Examination showed pseudobulbar paralysis of the tongue with no atrophy or fasciculation, a bilateral pyramidal syndrome in the limbs, and mild peroneal atrophy. EMG and muscle biopsy: denervation. MNCV: 45 $m \cdot s^{-1}$ (ulnar). CSF protein: 0.38 g/l. Nerve biopsy: normal.

The clinical data for the affected members of this family are summarized in Table 1 (Cases 32−43).

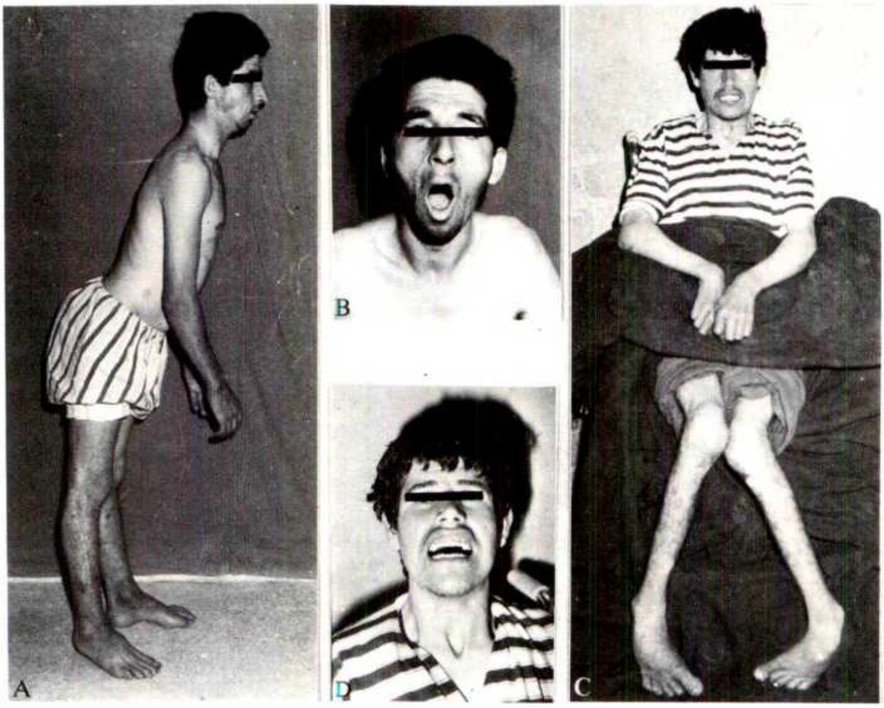

FIG. 6. A, *Case 41*, showing spastic stance; B, *Case 41*, complete paralysis of the tongue without atrophy or fasciculations; C, *Case 34*, spastic tetraplegia, with marked atrophy of legs and hands; D, *Case 34*, spastic pseudobulbar syndrome with paralysis of voluntary movement of the face.

RESULTS

The general clinical data are summarized in Table 2.

Group 1: upper limb (±bulbar) amyotrophy with a bilateral pyramidal involvement

The average age at onset was significantly older (17.6 yrs) for the isolated than for the familial cases (10.6 yrs) with no significant difference between the sexes. Bulbar involvement with atrophy and fasciculation of the tongue occurred in 1 familial case and 4 isolated cases. All cases studied are still living. The average time between onset and the date of the last clinical examination was 12 yrs (range 2−28 yrs). The mean

TABLE 1 CLINICAL FEATURES OF GROUP 3 CASES

Case	*Sex*	*Age (yrs)*	*Age at onset (yrs)**	*Onset signs*	*Pseudobulbar syndrome*	*Pyramidal syndrome*	*Amyotrophy of legs*	*MNCV ($m \cdot s^{-1}$)*	*SAP amp. (μV)*	*SCV ($m \cdot s^{-1}$)*	*CSF protein (g/l)*
32	M	60	10	pp	+++	b(50)	+	45 (uln)	–	–	0.38
33	F	36	6–7	pp	+++	++	–	–			
34**	M	27	3–4	sg	+++	b(12)	+hands	46 (med) 40 (per)	50 15	41 43	0.17
35	F	22	6–7	sg	++	++	+	75 (uln) 37 (per)	–	–	0.17
36	M	21	9	sg	++	++	+	–			
37	F	14	6–7	pp	+	++	–	–			
38	M	23	6–7	sg	++	++	–	62 (med)	–	–	0.44
39	M	28	3–4	pp	+++	b(20)	+	–			
40	F	32	7–8	pp	+++	+	+hands	47 (uln)	–	–	0.29
41	M	22	6–7	sg	++	++	–	–	–	–	0.22
42**	F	21	10	sg	++	++	–	50 (med) 38 (per)	50 18	48 44	0.26
43	M	7	6	pp	+	+	–	–			–

* Mean age 6.5. pp = pseudobulbar palsy; sg = spastic gait, +++ = severe; ++ = moderate, + = mild, b = bedridden, age when bedridden bracketed, ** = normal hexosaminidase A and B activity, MNCV = motor nerve conduction velocity, SAP amp = sensory nerve action potential amplitude, SCV = sensory conduction velocity, uln = ulnar, med = median; per = peroneal.

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TABLE 2 SUMMARY OF CLINICAL DATA

	M	*F*	*T*	*Age** *(yrs)*	*Age at onset** *(yrs)*	*Atrophy of hands*	*Peroneal atrophy*	*Bulbar syndrome*	*Pseudobulbar syndrome*	*CSF protein** *(g/l)*
Group 1	7	10	17	24.5 ± 3.9	14 ± 3	16	3	5	0	0.21 ± 0 13
Group 2	11	3	14	23.6 ± 3.8	14.2 ± 3.7	3	14	0	0	0.31 ± 0 21
Group 3	7	5	12	26 (7–60)	7.2 (3–20)	2	5	0	12	0.29 ± 0.11
Total	25	18	43	24 77 (7–60)	12.06 (3–25)	21	22	5	12	0.25 ± 0 06

Values in parenthesis represent ranges * Values = means ± 2 SEM. M = male; F = female; T = total.

CSF protein concentration was 0.21 ± 0.13 g/l. The genealogical data suggest autosomal recessive inheritance. This was supported by the normal clinical examination of the parents and the high frequency of consanguinity, which was present in 7 out of 10 siblings (the mean rate of consanguinity in Tunisia is 25–30%). It was confirmed by segregation analysis; the expected risk of 0.25 lay between the segregation ratios calculated by the sibship method ($P = 0.27$) and the Weinberg proband method ($P = 0.15 \pm 0.10$, $P < 0.5$). Biopsies from the superficial peroneal nerve were obtained in 12 cases. The results, included in our previous study (Ben Hamida *et al.*, 1987), revealed a non-significant reduction in the average density of small myelinated fibres (diameter < 8 μm) (patients: 8707 ± 1176, controls: 11316 ± 4077/mm^2) while the reduction in the total density was significant ($P < 0.05$) (patients: 10686 ± 1306, controls: 13351 ± 3015/mm^2). The reduction in the average density of the unmyelinated fibres of small diameter (diameter < 1 μm) (patients: 43937 ± 3033, controls: 64239 ± 31838/mm^2), as well as in the total density (patients: 50326 ± 4868, controls: 66306 ± 30426/mm^2), was not significant, whereas there was a highly significant increase ($P < 0.01$) in the large diameter unmyelinated fibres (diameter ≤ 1 μm) (patients: 6388 ± 3004, controls: 2067 ± 1717/mm^2).

Group 2: spastic paraplegia with peroneal atrophy

There was no bulbar involvement in this group; atrophy of the upper limbs with fasciculation was noted in 1 case. The pyramidal syndrome was present in the lower limbs in all cases, but with extensor plantar responses in only 4. The ankle jerks were absent bilaterally in 4 cases and unilaterally in 1. The average age at onset was 14.2 yrs (range 6–25 yrs). The average age at the date of examination was 23.2 yrs (range 14–38 yrs). The genealogical data suggest autosomal recessive inheritance. This was supported by the normal findings on examination of the parents. Consanguinity was present in 4 families out of 6. Segregation analysis confirmed autosomal recessive inheritance; the expected risk lay between the segregation ratios calculated by the sibship method ($P = 0.34$) and by the Weinberg proband method ($P = 0.23 \pm 0.13$, $P < 0.5$). The EMG revealed denervation in all cases, and motor nerve conduction velocity was within normal limits. The amplitudes of the sensory action potentials and sensory conduction velocity were normal in all cases studied. Superficial peroneal nerve biopsies were examined in 9 familial cases and in the 3 isolated cases, and showed no significant abnormalities. The average densities and distributions of the large myelinated fibres (diameter ≥ 8 μm) (patients: 1532 ± 481, controls: 2035 ± 1072/mm^2), small myelinated fibres (patients: 10270 ± 1304, controls: 11316 ± 4077/mm^2), small unmyelinated fibres (patients: 49889 ± 15189, controls: 64239 ± 31848/mm^2) and large unmyelinated fibres (patients: 3819 ± 2546, controls: 2067 ± 1717/mm^2) showed no significant differences in comparison with the controls. There was a moderate but a nonsignificant reduction in the average density of myelinated fibres (patients: 11803 ± 1169, controls: 13351 ± 3015/mm^2) and unmyelinated fibres (patients: 53709 ± 15310, controls: 66306 ± 30426/mm^2) comparable with that reported in the group 1 cases.

Group 3: spastic pseudobulbar syndrome with spastic paraplegia

A pseudobulbar syndrome and a spastic paraplegia constituted constant features in all patients in this group: atrophy of the legs was noted in 6 cases, and atrophy of the hands in only 2. There was no atrophy or fasciculation of the tongue.

The average age at onset was 6.5 yrs. The pseudobulbar syndrome, which was the initial symptom in 6 cases, was severe in 5, moderate in 5, and mild in the other 2. Spastic paraplegia, the initial symptom in 6 cases, had confined the patient to bed in 3 cases, was mild in 2, and moderate in 7. The EMG showed denervation in the 6 cases in which it was performed, and MNCV was within normal limits. SNCV and SAP amplitudes were normal in the 3 cases studied.

Examination of superficial peroneal nerve biopsies showed no significant abnormalities. The average densities and distributions of the large myelinated fibres (patients: 1779 ± 122, controls: 2035 ± 1072/mm^2), small myelinated fibres (patients: 8159 ± 2132, controls: 11316 ± 4077/mm^2), large unmyelinated fibres (patients: 8391 ± 6838, controls: 2067 ± 1717/mm^2) and small unmyelinated fibres (patients: 43004 ± 21632, controls: 64239 ± 31848/mm^2) showed no significant difference with the controls. The moderate reduction in the average densities of the myelinated fibres (patients: 9939 ± 2084, controls: 13351 ± 3015/mm^2) and the unmyelinated fibres (patients: 51396 ± 17883, controls: 66306 ± 30426/mm^2) was comparable with that reported in the group 1.

DISCUSSION

The absence of sensory disturbances and the purely motor nature of the distal limb deficits make it possible to group all our cases within the framework of the motor neuron diseases. Pyramidal involvement is attested to by the brisk and irradiating tendon reflexes, while anterior horn cell involvement is indicated by the distal amyotrophy, fasciculation, EMG evidence of denervation, normal MNCV, normal SAP amplitudes and normal or near normal findings on sensory nerve biopsy. This is quite distinct from the usual forms of progressive SMA, in which pyramidal involvement is lacking (Harding, 1984*b*; Ben Hamida *et al.*, 1988).

Group 1: upper limb (±bulbar) amyotrophy with spastic paraplegia

All the cases included in this category, whether familial (11) or isolated (6), presented a uniform clinical picture of juvenile ALS. The inheritance in the familial cases was autosomal recessive. The evolution of the disorder was very slow and benign. A bulbar and/or pseudobulbar syndrome sometimes completed the clinical picture. Comparable observations have been reported and classified within the hereditary spastic paraplegias (Refsum and Skillicorn, 1954; Silver, 1966; Cross and McKusick, 1967). Rarely, they have been classified with juvenile ALS (van Bogaert, 1925; Emery and Holloway, 1982; Ben Hamida and Hentati, 1984). The concept of very slowly progressive juvenile or infantile onset ALS has not been generally adopted, perhaps because of the rarity of these cases and clinical differences from the usual sporadic cases of adult-onset ALS.

As early as 1905, Holmes described observations of 2 sisters aged 15 and 13 yrs, born of healthy parents who were first cousins. The elder had nasal speech, amyotrophy of the hands and legs, pes cavus, brisk and irradiating tendon reflexes, and extensor plantar responses. In addition, nystagmus was present. The picture was the same in the younger sister, but without nystagmus. These 2 cases are very similar to our own observations on 2 sisters (Cases 5 and 6, family 3). In 1954, Refsum and Skillicorn reported a 12-member sibship, 3 of whom had, between the ages of 3 and 5 yrs, developed slowly progressive paraparesis, with diffuse amyotrophy and fasciculation, as well as bulbar disturbances and skeletal deformities. The authors related this picture to that of ALS, and alluded to an autosomal recessive transmission. Much more similar are the observations on 2 brothers made by Gragg *et al.* (1971), and the observations of Myllylä *et al.* (1979), in which a pseudobulbar syndrome completed a clinical picture that could be described as juvenile ALS.

The 20 cases reported by Cross and McKusick (1967) as the Troyer syndrome were characterized by spastic paraparesis associated with distal amyotrophy, beginning in early childhood and confining the patients to bed by their third or fourth decade. There was an accompanying pseudobulbar syndrome, and in some cases choreoathetosic movements and cerebellar signs. Such a picture is clearly quite different from that of pure juvenile ALS. The same is true of the large series of Bouchard *et al.* (1978) in which there were objective sensory disturbances and histological changes in sensory nerves.

Among descriptions of cases with autosomal dominant transmission are the families reported by Silver (1966) in which neurological disturbances began, in the 12 affected subjects, between the ages of 15 and 30 yrs, associating amyotrophy of the small muscles of the hand, a pyramidal syndrome of the lower limbs, and amyotrophy of the legs. In 3 cases, there was impairment of vibration sense. Transmission was autosomal dominant, as in the 18 observations belonging to a family reported later by van Gent *et al.* (1985) in which the older subjects showed some sensory disturbances.

Group 2: spastic paraplegia with peroneal muscular atrophy

In addition to the commoner type in which amyotrophy is mainly confined to the hands, there is an autosomal recessive form in which spastic paraplegia predominates, associated with peroneal atrophy and, to a lesser extent, with atrophy of the hands. Discussion of these cases has been based on hereditary spastic paraplegia (HSP) which, in the 'pure form' does not include distal atrophy. Nevertheless, Harding (1984*a*) discussed certain cases from the literature in which atrophy may appear. Mention has occasionally been made of peroneal atrophy (of the distal SMA type) arising during the course of spastic paraplegia (Maas, 1904; Garland and Astley, 1950). The observations we report here are completely different from the Charcot-Marie-Tooth (CMT) type of peroneal muscular atrophy associated with a pyramidal syndrome, designated hereditary motor and sensory neuropathy type V by Dyck (Dyck and Lambert, 1968; Dyck, 1984), which has been well analysed in a recent review (Harding and Thomas, 1984). In this study, involving 25 individuals, the illness was clearly a sensorimotor neuropathy of the CMT type, associated with pyramidal signs (and with autosomal dominant transmission). Sensory disturbances were evident clinically and electrophysiologically. This differs from our own patients who showed no objective sensory disturbance and in whom the sensory nerve studies showed insignificant changes similar to those mentioned in cases of typical juvenile ALS (Ben Hamida *et al.*, 1987), unlike what was reported by Dyck and Lambert (1968) and by Dyck (1975, 1984). Our study in fact concerns a pure motor type of peroneal muscular atrophy, consistent with lesions of the anterior horn cells of the thoracolumbar cord, with accompanying involvement of the pyramidal system. Given the familial nature of our cases and the electrophysiological and histopathological arguments, we consider that such a clinical picture can best be designated as a rare type of juvenile ALS. In the group 1 cases, the amyotrophy of the small muscles of the hand reflects an anterior horn cell involvement in the cervical cord, while in these group 2 cases, also purely motor, the amyotrophy, which is essentially peroneal, corresponds to involvement of the lumbosacral anterior horn cells, and is associated with a spastic paraplegia without bulbar signs. The severity of the lower motor neuron involvement in the lumbosacral cord could explain the abolition of the ankle jerks and the failure to obtain a plantar response at times.

Group 3: spastic pseudobulbar syndrome with spastic paraplegia

The appearance of an isolated, slowly progressive paraplegia or tetraplegia led Charcot (1865), Charcot and Joffroy (1869) and Erb (1875) to discuss the possibility that this might be a variant of ALS in which involvement of the anterior horn cells had remained undetectable. Thus primary lateral sclerosis could be considered to be a rare clinical form of ALS (Beal and Richardson, 1981; Russo, 1982). In his extensive review, Fisher (1977) distinguished four primary lateral sclerosis syndromes, which were recently discussed by Gastaut *et al.* (1988). Within this framework it is interesting to note the description of two syndromes called ‘chronic progressive spastic bulbar paralysis’ and ‘chronic progressive bilateral spinobulbar spasticity’, which affect only middle-aged or elderly patients. Gastaut *et al.* (1988) classified their 5 examples of chronic progressive spinobulbar spasticity as a rare form of primary lateral sclerosis. The 12 patients we report here show similarly, but at an infantile and a juvenile age, a chronic spastic pseudobulbar syndrome with spastic tetraplegia which is entirely comparable with what Fisher (1977) and Gastaut *et al.* (1988) termed chronic progressive spinobulbar spasticity. What sets them apart is the familial character of our cases, and the associated peroneal amyotrophy and, more rarely, atrophy of the hands, without sensory disturbances. The disease is very slowly progressive; transmission seems to be autosomal recessive. The appearance of muscle atrophy indicates involvement of the anterior horn cells, and constitutes a firm argument in favour of classifying this condition within the juvenile ALS group as a rare and particular expression of it.

CONCLUSIONS

The existence of slowly, progressive, benign infantile and juvenile motor system disease (chronic juvenile ALS) is indisputable. Whether the clinical picture is that of juvenile ALS comparable with classical Charcot's disease, or whether it simulates hereditary spastic paraplegia with accompanying anterior horn cell involvement or primary lateral sclerosis but with peroneal atrophy, in either case the clinical signs indicate a combined disorder of the lower and upper motor neurons. This is supported by neurophysiological investigation and histopathological studies on sensory nerve biopsies. This has made it possible to eliminate a diagnosis of uncomplicated SMA and of CMT disease with an accompanying pyramidal syndrome.

Infantile and juvenile ALS is a rare condition. Its authenticity has been difficult to establish because of the lack of any comprehensive study of a large series, as in the present report. These disorders seem to be much more frequent in this geographical region. The high frequency of consanguinity in Tunisia could play an important role. The disease is most often familial and, in our experience, transmission is autosomal recessive. More detailed genetic studies on the three syndromes that we have described will make it possible to specify more precisely the relationships between them and with the other degenerative diseases that affect the motor system.

In recent years there have been descriptions of cases of juvenile onset with clinical features that resemble ALS but which usually show multisystem involvement and in which underlying hexosaminidase deficiency has been established (e.g., Mitsumoto *et al.*, 1985). Although hexosaminidase activity was not studied systematically in the present series of cases, in those in which it was examined, normal values were obtained.

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DURATION AND SELECTIVITY OF BLOOD-BRAIN BARRIER BREAKDOWN IN CHRONIC RELAPSING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS STUDIED BY GADOLINIUM-DTPA AND PROTEIN MARKERS

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SUMMARY

Gadolinium-DTPA (Gd-DTPA) enhancement seen with magnetic resonance imaging in chronic relapsing experimental allergic encephalomyelitis (CREAE) corresponded with sites of blood-brain barrier breakdown judged by traditional markers in areas of inflammatory demyelination. Duration of Gd-DTPA leakage for individual lesions in CREAE varied from 5 days to more than 5 wks. By contrast, in acute EAE leakage was of shorter duration (always less than 5 days). Selective enhancement was observed in CREAE lesions using Gd-protein markers. Gd-albumin enhancement was not always seen in areas of leakage of the smaller molecular weight compound Gd-DTPA. The addition of immunoglobulin to the gadolinium complex led to enhancement of lesions not seen with Gd-albumin alone. From the similarities between the histology and the patterns of Gd-enhancement in CREAE and multiple sclerosis, it is probable that Gd-enhancement reflects active inflammation (with or without demyelination) in the human disease.

INTRODUCTION

In multiple sclerosis (MS), lesions seen with magnetic resonance imaging (MRI) frequently enhance with gadolinium-DTPA (Gd-DTPA) (Grossman *et al.*, 1986; Gonzalez-Scarano *et al.*, 1987; Kermode *et al.*, 1988; Miller *et al.*, 1988). What this breach in the normal blood-brain barrier to Gd-DTPA means in histological terms is of considerable interest because of its implications for understanding the development and evolution of the plaque and its potential use in monitoring treatment. There are important histological similarities between the lesions in MS and chronic relapsing experimental allergic encephalomyelitis (CREAE) in guinea-pigs (Lassmann, 1983). In the latter there is focal breakdown of the blood-brain barrier, but the use of traditional markers of barrier breakdown has precluded the establishment of the time course in individual lesions (Kristensson and Wiśniewski, 1977; Grundke-Iqbal *et al.*, 1980). Here we report a serial study of the evolution of blood-brain barrier changes in EAE using

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Gd-DTPA, in which we have related the functional and the histological changes and have compared both with those obtained using traditional methods. Abstracts of some aspects of this work have been published (Hawkins *et al.*, 1988*a*, *b*).

METHODS

Forty guinea-pigs with CREAE were studied. CREAE was induced in juvenile (17–22 day old) strain 13 guinea-pigs by two 0.1 ml subcutaneous injections into the dorsa of the hindfeet of an inoculum containing 50% (w/v) homogenate of guinea-pig spinal cord in phosphate-buffered saline (PBS), added to an equal volume of complete Freund's adjuvant (FCA) containing 10 mg/ml Mycobacterium tuberculosis (Difco H37Ra). Acute EAE was induced in 10 adult Hartley guinea-pigs by the same method. Adult animals were chosen because of the larger size spinal cord for imaging purposes and usually a more severe disease course than juvenile animals. Ten guinea-pigs (6 strain 13 and 4 Hartley) received only an emulsion of 1:1 (w/v) PBS/CFA and were studied as control animals for MRI and histological examination. CFA-injected animals were chosen as controls following the observation of increased protein leakage across the blood-cerebrospinal fluid barrier after injection of Freund's adjuvant (Reiber *et al* , 1984). Strain 13 controls were studied between 2 and 6 months after CFA injection and Hartley guinea-pigs at 10 and 14 days after injection, as control animals for CREAE and acute EAE, respectively. Animals were weighed and clinical assessment (on a 5-point scale) recorded daily.

Gd-DTPA (Schering AG) was injected intravenously in a dose of 0.25 mmol/kg. Gd-protein complexes were synthesized by a method previously described (Kornguth *et al.*, 1987). Gadolinium was combined either to albumin alone, or to immunoglobulin with albumin. Using this method it has been shown that approximately 10 Gd-DTPA molecules are combined to each protein molecule. The albumin used was bovine serum albumin (Sigma); the immunoglobulin was mouse monoclonal IgG without known specific binding property (IgG3 ascites myeloma protein, Sigma). Relaxivity of Gd-protein complexes ranged from 30 to 60 $L \cdot mmol^{-1} \cdot s^{-1}$. These values were calculated from T1 relaxation times of the preparations at different concentrations (at 20° C), calculated from a multislice inversion recovery sequence (IR2000/500/40) and spin echo sequence (SE2000/40) using a 0.5 Tesla Picker International imager and an algorithm supplied by the manufacturer. The accuracy of calculating relaxation times in this way has been validated using the imager as a spectrometer (Johnson *et al.*, 1987). In order to compare enhancement on scans resulting from equivalent concentrations of two different contrast agents when injected, solutions of contrast agents were prepared having the same relaxation rate by appropriate dilution in sterile water, and equal volumes of the prepared solutions were injected. This was necessary because the compounds used have differing ratios of attached Gd and differing molecular size leading to different relaxivity.

MRI was performed using a 0.5 Tesla machine and specially designed animal gradient coils as required (Picker International). Lower thoracic and lumbar spinal cord was routinely scanned in transverse (axial) plane with 2 mm slice thickness and 10 cm field of view (256 values of phase encoding gradient with pixel size 0.4 mm × 0.4 mm). Specially built surface receiver coils were used to maximize signal-to-noise ratio.

For imaging, both spin echo and inversion recovery scans were used. The usual sequences were (1) a T2-weighted spin echo (SE2000/80 and 2000/120, 12 slices, 4 repetitions over 35 min) for identification of lesions without Gd-DTPA, and (2) a T1-weighted spin echo (SE560/40, 4 slices, 8 repetitions over 20 min) which was repeated serially to observe the appearance and disappearance of enhancement For serial study of enhancement of selected lesions in more detail, at an improved contrast-to-noise ratio, an inversion recovery sequence was used (IR560/300/40, 2 slices, 4 repetitions over 10 min).

Anaesthesia for MRI was induced by a Hypnorm/Hypnovel mixture (1 part fentanyl-fluanisone 0.32 mg/ml – 10 mg/ml, 1 part midazolam 5 mg/ml and 2 parts sterile water) in a dose of 8 ml/kg by intraperitoneal (i.p.) route, and an additional 4 ml/kg after 1 h was found a convenient method of prolonging anaesthesia for up to 3–4 h. Animals were wrapped in cotton wool before scanning and it was found that rectal temperature measured with a clinical thermometer was stable between 36° and 37.5° C during the scan.

Images were obtained at different stages of chronic and acute EAE. Three animals (strain 13) with a chronic relapsing course having entered a progressive phase of disease were scanned serially with Gd-DTPA two or three times per week for a period of 5 wks or more in order to establish the duration of lesion enhancement with Gd-DTPA. Two animals (strain 13) were scanned serially through acute EAE into their first relapse of CREAE. Two animals (strain 13) were scanned serially from their first relapse of CREAE when Gd-DTPA enhancement was seen through to development of lesions on the unenhanced T2-weighted spin echo images. Three Hartley guinea-pigs showing Gd-DTPA enhancement in acute EAE were followed by alternate day scanning to determine the duration of lesion enhancement. Six control animals were studied (4 strain 13 and 2 Hartley).

For the study of lesions using Gd-protein conjugates, areas of the cord were always chosen where enhancement with Gd-DTPA had been shown to occur. Four animals with CREAE in the progressive phase of illness were studied with Gd-albumin, which was injected intravenously (i.v.) between 3 and 6 h after Gd-DTPA injection (when Gd-DTPA enhancement had resolved). The dose of albumin for this experiment was 50 mg/kg. Four additional CREAE animals in the progressive phase were studied where lesions showed enhancement with Gd-immunoglobulin after i.v. injection, and enhancement was compared with Gd-albumin alone. Gd-albumin was injected between 4 and 8 h after Gd-immunoglobulin (when Gd-immunoglobulin enhancement had resolved). The dose of immunoglobulin was 0.5 mg/kg (with 10 mg/kg albumin), and the dose of albumin when Gd-albumin alone was injected, 10 mg/kg.

The results of MRI were assessed by comparison with images from control animals (C.P.H.) and scans were reviewed blindly (E P.G.H du B.). Only when areas of enhancement were thought definite (rather than possible or suspicious) by both observers were they included in the study.

Spinal cord samples from 15 guinea-pigs showing Gd-enhancement on MRI, representing different stages of EAE, were examined by light and electron microscopy. In order to determine whether Gd-enhancement preceded histological changes of EAE, these included 2 animals with acute EAE (Hartley) (examined within 24 h), and 4 animals with CREAE examined within 48 h of the first appearance of Gd-enhancement on the scan, established by serial scanning. All animals were perfusion-fixed via the ascending aorta with a mixture of 3% glutaraldehyde and 1% paraformaldehyde unless otherwise stated. By reference to the position of adjacent vertebral bodies visualized in sagittal MRI pilot scans, care was taken to ensure that the 2 mm cord sections for histology corresponded with the 2 mm slices averaged on MRI. Following primary fixation, tissue samples were washed in 0.1 M buffer, vibratome sectioned (50–200 μm), postfixed in 1% aqueous osmium tetroxide, and dehydrated through ascending concentrations of ethanol. The wash buffer was that employed as vehicle in the primary fixative solution (except when perfusing with lanthanides as described below)

Changes in the permeability properties of the blood-brain barrier were assessed in 6 animals (3 with CREAE, 1 with acute EAE and 2 control animals—1 strain 13 and 1 Hartley) using the horseradish peroxidase (HRP) technique (MW 40 kDa) (Kristensson and Wiśniewski, 1977; Kitz *et al.*, 1984); 250 mg/kg of Sigma type II HRP was administered by means of intracardiac injection in sterile saline 30–60 min before perfusion-fixation (via the aorta with 1.5% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M sodium phosphate buffer, at pH 7.4 and room temperature) All injections of HRP were preceded by the i.p. administration of an antihistamine (diphenhydramine). In order to counter the inhibitory effects of fixatives on HRP activity, spinal cord segments were transferred to 0.1 M sodium phosphate within 1 h of perfusion. HRP activity was developed by incubating vibratome sections for 30 min in a medium comprising 7 mM 3,3′-diaminobenzidine, 0.05 M Tris-HCl and 0 01% hydrogen peroxide as substrate at pH 7.6 and room temperature. After examination by light microscopy and photography, sections were osmicated and processed for plastic sectioning

Lanthanum and gadolinium nitrates (gadolinium is one of the lanthanides) (Aldrich Chemical Company, Dorset) were used as smaller molecular weight markers of blood-brain barrier breakdown. In these latter cases, tissue sections from 4 animals (2 CREAE and 2 strain 13 control animals) were perfusion-fixed via the aorta with 1% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M cacodylate buffer together with 1% (w/v) lanthanide nitrate, at an acid pH of 6.9 and room temperature, and 2 mm sections were transferred to 0.1 M sodium phosphate at an alkaline pH of 7.6 and 4° C in order to promote the in situ deposition of insoluble lanthanide phosphate (Shaklai and Tavassoli, 1977).

Semithin sections cut with glass knives were stained with 1% alcoholic toluidine blue and ultrathin sections were cut with a diamond knife and stained by sequential 10 min immersions in saturated ethanolic uranyl acetate followed by Reynold's lead citrate at room temperature. A Reichert OM3 ultramicrotome was used in each case. Stained and unstained sections were examined in Jeol 100CX and 1200EX electron microscopes operating at 80 kV and images recorded onto Kodak 4489 electron image film. A Leitz orthoplan light microscope was used to record phase contrast and Nomarski images of unstained sections and brightfield images of stained sections on Kodak plus X pan film.

Energy dispersive x-ray microanalysis of suspected lanthanide deposition was undertaken using a mark 10 ASID STEM unit and a Link Analytical AN 10 85/S system fitted to a Jeol 1200EX microscope operating at 80–100 kV.

RESULTS

CREAE was characterized by an initial acute episode of paralysis with weight loss developing 10–14 days after inoculation (acute EAE) from which most animals usually recovered to enter a chronic phase of disease some 6 wks later. Chronic disease presented usually as a relapsing/remitting illness with either good recovery or a general underlying progression of disability. With time a progressive course was often dominant and some animals followed a steady decline from the first relapse.

Magnetic resonance imaging

When 6 control animals were studied by MRI no areas of abnormally high signal were seen in the spinal cord on unenhanced T2-weighted scans. Following injection of Gd-DTPA there was always enhancement of the spinal meninges but no areas of enhancement affecting the parenchyma of the cord.

When CREAE animals were studied lesions were often visible on unenhanced T2-weighted spin echo images of lower thoracic and lumbar cord, usually from 3 months after inoculation (fig. 1A, arrow) as has been previously described (Karlik *et al.*, 1987). Lesions were mainly situated ventrolaterally in the cord or dorsolaterally. Many of the lesions showed Gd-DTPA enhancement on T1-weighted sequences (fig. 1B, arrow). Sometimes enhancement involved a smaller area of the cord than the abnormal signal on the unenhanced scan.

Areas of Gd-DTPA enhancement with no detectable lesion on unenhanced T2-weighted scans could be seen at an earlier stage, often from 2 months after inoculation, and on several occasions enhancement was seen to occur before the onset of the clinical features of relapse. Enhancement was usually seen ventrolaterally or dorsolaterally but often affected the lateral border of the cord. Maximum enhancement with Gd-DTPA usually occurred 15 to 35 min after injection, but some enhancement could persist for several hours.

When different groups of CREAE animals were studied, most Gd-enhancement was seen to affect the spinal cord of animals entering a progressive phase of illness and this group was chosen to study the duration of lesion enhancement and for comparative

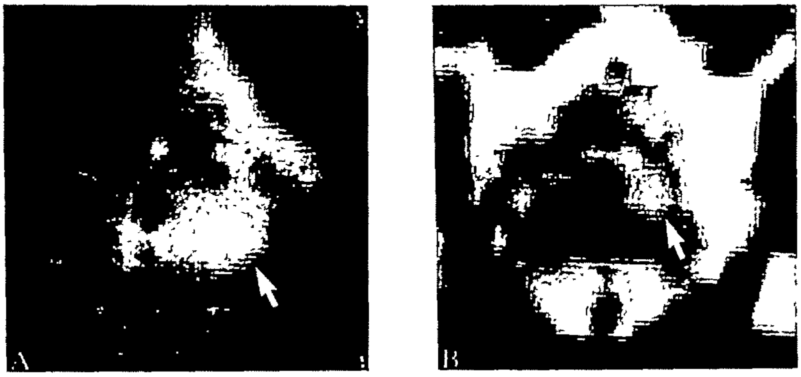

Fig. 1. A, unenhanced T2-weighted scan (SE2000/80). Transverse slice of lower thoracic cord showing an area of abnormally high signal affecting the ventrolateral cord (arrow). B, enhanced T1-weighted scan (IR560/300/40) of the same slice as shown in A, but following injection of Gd-DTPA An area of abnormal enhancement is seen in the ventrolateral cord (arrow) corresponding to the area of abnormal signal seen on the unenhanced scan in A; enhancement in this case involves a smaller area of the cord than the abnormality on the unenhanced scan.

study of different Gd-DTPA compounds. When animals with relapsing and remitting diseases (rather than progressive disease) were imaged, Gd-DTPA enhancement was seen at the time of relapse, but this was of more limited extent than in animals with progressive disease. Animals with inactive clinical disease showed little or no abnormality.

Results from 3 animals with CREAE studied serially with Gd-DTPA to determine the duration of lesion enhancement, and from 3 animals showing areas of Gd-DTPA enhancement in acute EAE, are shown in fig. 2. Lesions studied in CREAE enhanced

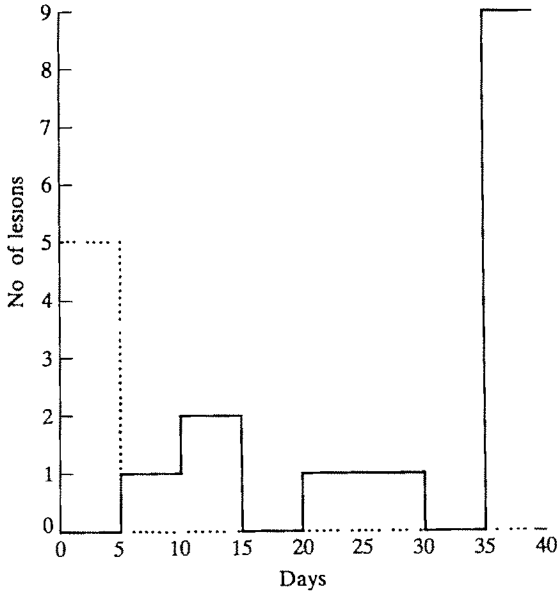

Fig 2. Duration of enhancement with Gd-DTPA for individual lesions is shown for chronic and acute EAE (3 animals in each group), established by serial scanning. Dotted lines = acute EAE, continuous line = chronic EAE

for periods of from 5 days to more than 5 wks, and 9 out of 14 lesions in progressive disease were still enhancing after 5 wks study. Some lesions enhanced for only 1, 2 or 3 wks. In contrast, leakage in acute EAE was shorter and always lasted less than 5 days. In animals with CREAE there were three occasions when recurrence of enhancement was seen to occur in the same area of a spinal cord previously showing enhancement.

Unenhanced T2-weighted images did not always show detectable lesions in areas where Gd-DTPA enhancement was observed. Of the lesions in CREAE with a shorter duration of enhancement (less than 4 wks) and in acute EAE, no definite lesion could be detected on T2-weighted scans. Of the lesions in CREAE with a longer duration of enhancement (greater than 5 wks), 3 of 9 were seen on the unenhanced T2-weighted scans.

Enhancement following i.v. injection of Gd-protein conjugates showed a different time course from that of Gd-DTPA; maximum enhancement did not usually occur until 35 min after injection and sometimes not until 55–75 min. When 6 lesions in 4 animals were studied, all showing enhancement with Gd-DTPA, only 4 showed detectable enhancement with Gd-albumin, and in 2 of these lesions the extent and intensity of enhancement was less than with Gd-DTPA. Of the 6 lesions showing enhancement with Gd-immunoglobulin, 2 could not be seen with Gd-albumin alone and in a further lesion the extent of enhancement wiht Gd-albumin was less than with Gd-immunoglobulin.

Histology

On routine histology control animals showed no abnormality by light or electron microscopy. When animals with CREAE were examined histologically at different stages after the development of MRI lesions there was always perivascular and often confluent inflammation with demyelination at the sites of Gd-enhancement. There was no occasion in CREAE or acute EAE when Gd-DTPA enhancement was found in the absence of perivascular inflammation. Areas of confluent inflammation were sometimes present in the cord where Gd-enhancement had not been detectable on the 2 mm MRI slice, but when this occurred the inflammatory changes usually involved smaller areas of the cord compared with the areas of confluent inflammation seen where Gd-enhancement had been detectable on the scan.

When control animals were studied with HRP, reaction product was seen in the meninges but not the parenchyma of the spinal cord. When EAE animals were studied, the greatest concentration of reaction product in vibratome sections corresponded with areas of Gd-DTPA and Gd-protein enhancement at MRI (fig. 3, arrows). There were, however, areas of less intense HRP staining affecting small areas of some vibratome sections where the 2 mm MRI slice had not shown detectable Gd-enhancement. Reaction product was observed most frequently in relation to ventrolateral penetrating vessels of the cord. On light microscopy HRP was mainly perivascular; in 1 animal with CREAE diffuse perivascular leakage was seen, and in the others a particulate distribution, with product mainly in perivascular phagocytes (fig. 4, large arrows). On electron microscopy reaction product was present in the perivascular space, particularly around venules and small veins (greater than 10 μm diameter). An increased number of endothelial vesicles

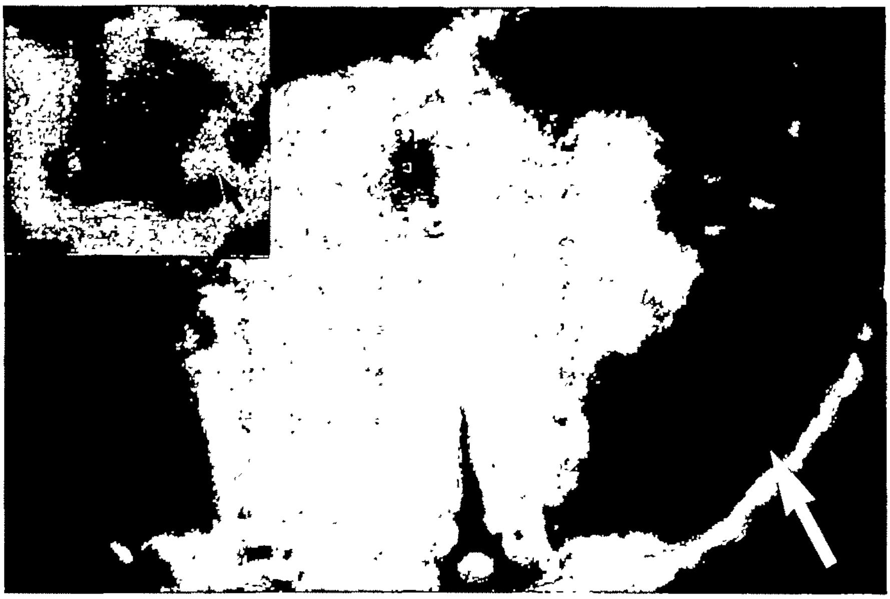

Fig 3. Vibratome section showing dense HRP product in the ventrolateral cord (large arrow) corresponding to an area of Gd-enhancement affecting the ventrolateral cord at MRI (*inset*, small arrow) (SE560/40).

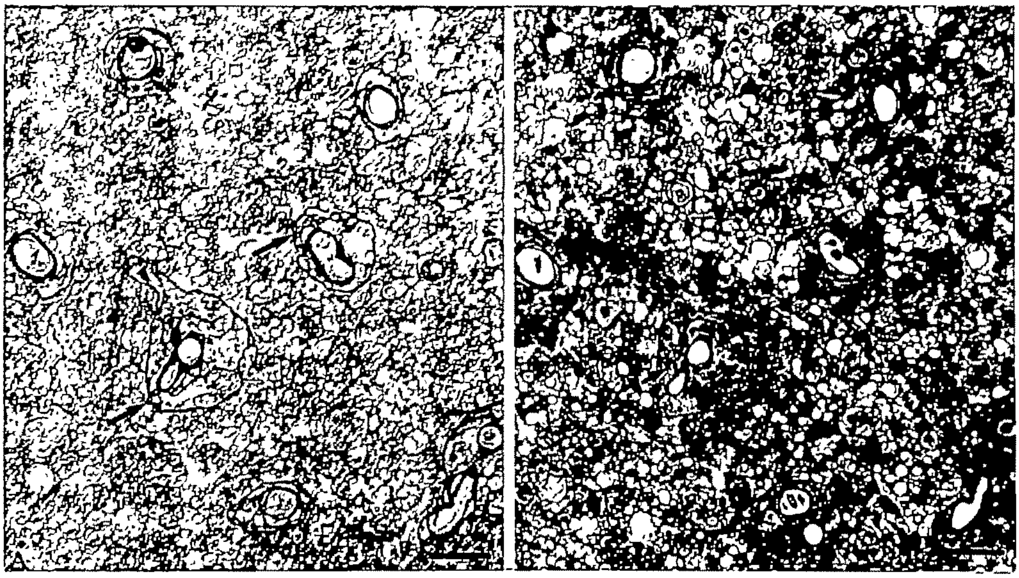

Fig 4. Light microscopy from an area of HRP leakage (shown by arrow) on vibratome section in fig. 3. A, unstained section using Nomarski optics showing rings of HRP leakage from small vessels (large arrows) B, adjacent serial section stained with toluidine blue demonstrating perivascular inflammation (small arrows) associated with HRP leakage, and accompanying demyelination (arrowheads). Bars = 20 μm.

containing reaction product was observed when compared with control animals and there was often thickening of the endothelial wall in areas of greatest HRP leakage. No example of a continuous track of HRP connecting the vascular lumen with perivascular space between the endothelial cells was observed to suggest that there had been opening of interendothelial tight junctions. Inflammatory cells were often seen adherent to the luminal surface of the endothelial cells lining vessels from areas of the cord showing the most leakage (fig. 5).

When control animals were perfused with lanthanum or gadolinium nitrate and studied in unstained and stained sections by electron microscopy, an electron-dense deposit was present a long the luminal surface of the endothelial cells, probably representing anionic binding sites (Nagy *et al.*, 1981). Deposits of gadolinium were seen around meningeal vessels, but no deposit was seen in the perivascular space of the cord. When CREAE animals were examined in areas of the cord showing Gd-enhancement at MRI, an increased number of electron-dense endothelial vesicles was seen when compared with control animals. Lanthanum or gadolinium, depending on which has been injected, was identified in the vesicles by energy dispersive x-ray microanalysis at electron microscopy (fig. 6, arrows). A dense deposit was often present in the perivascular space. Interendothelial junctions appeared closed. Fig. 7 (arrow) shows an electron-dense deposit

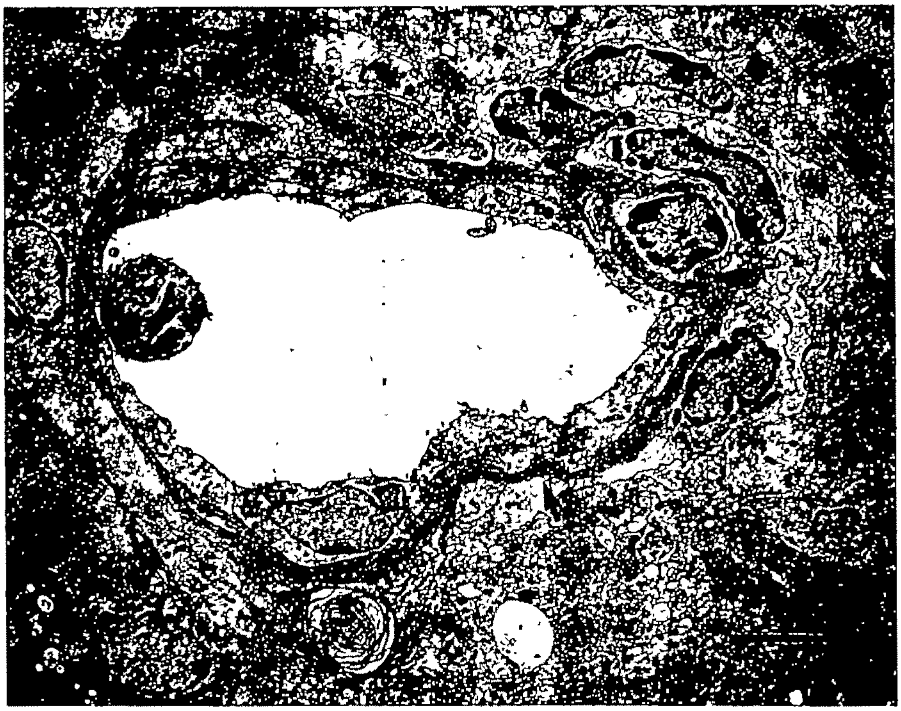

FIG. 5 Electron micrograph from the area of HRP leakage seen in fig. 4, showing a venule with electron-dense HRP in the perivascular space (arrow) and an inflammatory cell adherent to the luminal surface of the endothelium. Bar = 5 μm.

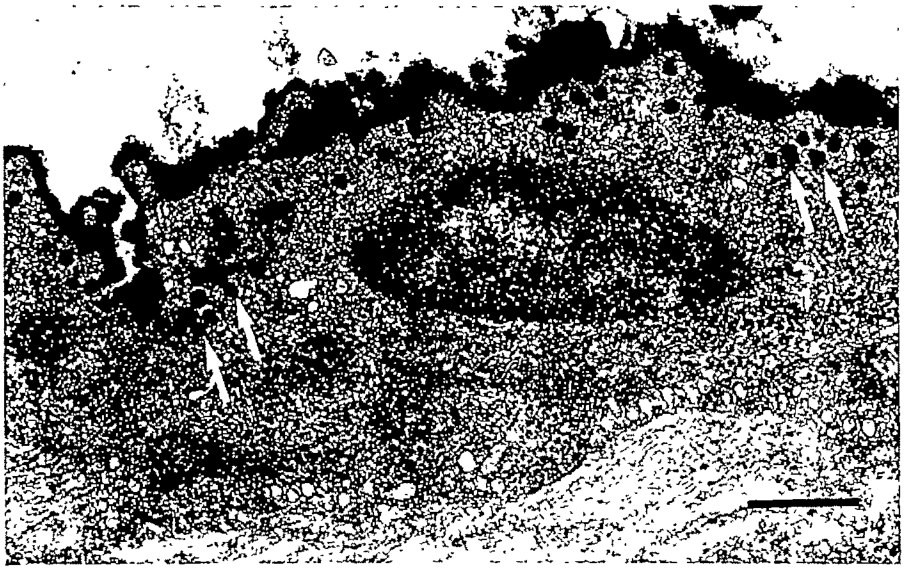

FIG 6 Electron micrograph demonstrating endothelial vesicles containing lanthanum (arrows) in an area of the cord which had shown Gd-enhancement with MRI following which lanthanum nitrate had been perfused Bar = 0 5 μm.

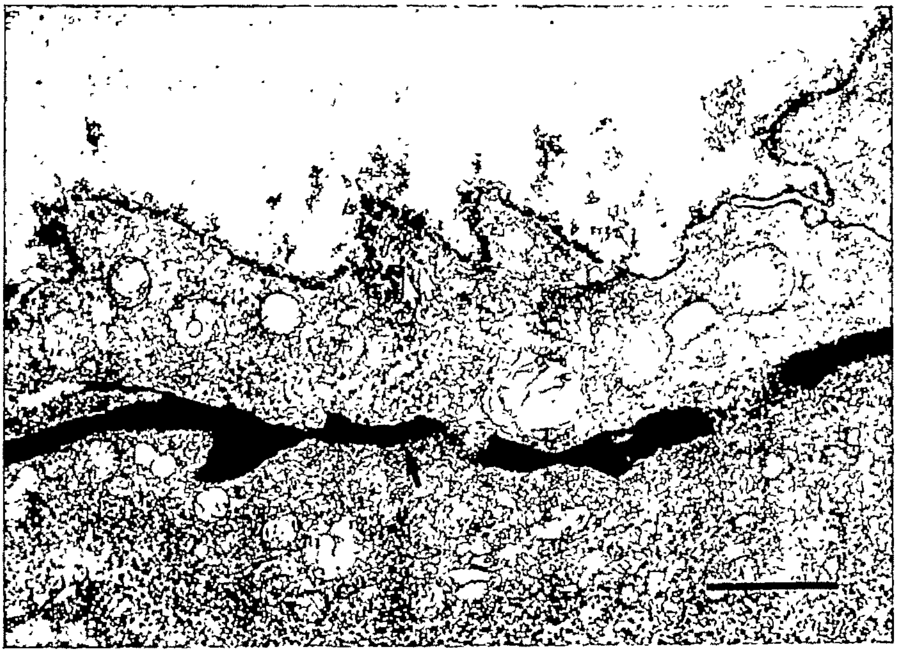

FIG 7 Electron micrograph showing a dense deposit of Gd in the perivascular space (black arrow) and vesicles containing Gd in the cytoplasm (white arrows) following perfusion with gadolinium nitrate, in an area of the cord which had shown Gd-enhancement with MRI Bar = 1 μm.

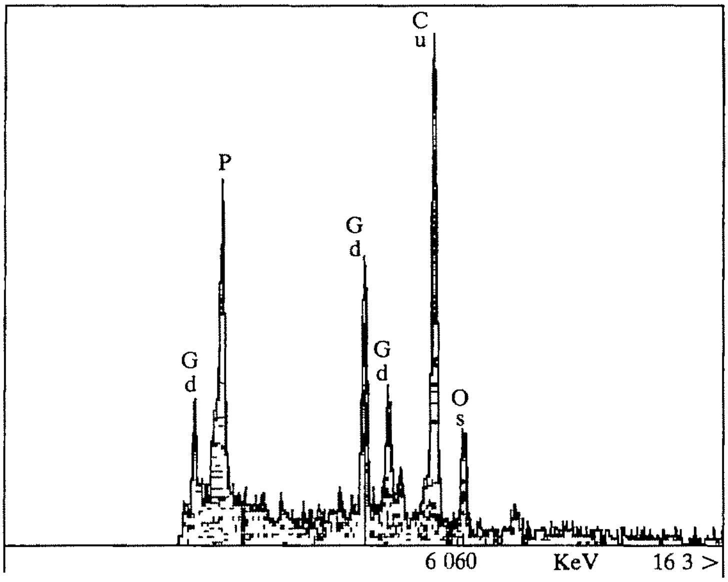

FIG 8. Energy dispersive x-ray microanalysis spectrum obtained on electron microscopy from the perivascular space shown in fig. 7, confirming the deposit to be gadolinium phosphate (Gd = gadolinium, P = phosphorus, Cu = copper from the electron microscope grid, Os = osmium from fixation and KeV = KiloelectronVolts).

in the perivascular space following perfusion with gadolinium nitrate of an area of the cord which had shown Gd-enhancement on scan, and the deposit was identified as gadolinium phosphate by x-ray microanalysis (fig. 8).

The results of a detailed light and electron microscope study of 12 CREAE lesions at different stages in 12 animals were reviewed. The lesions chosen for study had all shown Gd-DTPA leakage on MRI, but only 4 of them had been seen on an unenhanced T2-weighted image. In these latter 4 lesions the degree of inflammation was graded only as mild or moderate, and never involved a large area. Lesions showing inflammatory change over large areas (4 animals) were not detected on unenhanced T2-weighted scans. Other histological features (demyelination and gliosis) showed no relationship to the presence or absence of lesion on unenhanced scan.

DISCUSSION

This study has shown that areas of Gd-DTPA and Gd-protein enhancement seen with MRI corresponded with areas of blood-brain barrier breakdown judged by traditional barrier markers in CREAE. There was minor HRP leakage seen on vibratome sections not accompanied by Gd-enhancement on the scans, but absolute correlation would not be expected when comparing two compounds with different molecular weights (HRP MW 40 kDa; Gd-DTPA MW 550 Da). The mechanism of production of an area of abnormal signal on the scan also needs to be taken into account: the MR image is derived from a signal averaged over 2 mm slice thickness and a small area of leakage affecting one vibratome section (0.2 mm thick) could become insignificant with averaging

The duration of leakage for individual spinal cord lesions was established by serial Gd-DTPA scanning. In animals with CREAE in a progressive phase of illness, although many lesions (9 out of 14) continued to enhance for more than 5 wks, often the duration of enhancement was shorter, lasting only a few weeks and this would have been missed using traditional histological blood-brain barrier markers. Recurrence of enhancement in the same area of a spinal cord previously showing enhancement was seen in CREAE. Enhancement of previously nonenhancing lesions has been described in MS (Miller *et al.*, 1988).

Lesions in CREAE were sometimes seen with Gd-DTPA in the absence of a detectable lesion on the standard unenhanced T2-weighted image, particularly in the early stages of evolution of the condition. Lesions on unenhanced T2-weighted scans in EAE and MS are thought to be visible because of the presence of an increase in free tissue water associated with the lesion, leading to an increase both in T1 and T2 relaxation times. It is possible that lesions in the early stages of EAE are only clearly seen on T2-weighted images if the accompanying inflammatory infiltrate of the lesion is mild or moderate, and not extensive, in comparison with the increase in free tissue water (oedema) in the lesion. This is because protein is known to have a marked T2 shortening effect in this context, as shown during the acute phase of protein leakage in vasogenic oedema before protein resorption (Barnes *et al.*, 1987), and inflammatory infiltrate with protein exudation would tend to oppose the increase in T2 and T1, caused by an increase in tissue water. This mechanism has already been suggested for 'normalization' of in vitro NMR parameters in acute EAE (Karlik *et al.*, 1986).

There are varying reports of MRI abnormality seen on unenhanced sequences in acute EAE. In primates and dogs an abnormal signal can be clearly seen on unenhanced T2-weighted spin echo scans (Stewart *et al.*, 1985; Kuharik *et al.*, 1988). In guinea-pigs with acute EAE, in one series, only 1 animal out of 27 studied showed a definite lesion on unenhanced MRI (2 cm slice thickness) of the brain (Simon *et al.*, 1987), but Grossman *et al.* (1987) reported frequent lesions on T2-weighted sequences (using 3 mm slice thicknesses), with accompanying perivascular inflammation on histology.

Gd-DTPA enhancement in acute EAE was always accompanied by perivascular inflammation, and in CREAE by perivascular inflammation and demyelination histologically. Since the demyelination induced by cuprizone (which is not accompanied by inflammation) is unaccompanied by blood-brain barrier breakdown as judged by traditional markers (Bakker and Ludwin, 1987), we conclude that Gd-enhancement is reflecting the inflammation and not the demyelination per se. Gd-enhancement of cerebral lesions in acute EAE in dogs has recently been described and on histology perivascular inflammation was associated wtih Gd-enhancement (Kuharik *et al.*, 1988).

The mechanism of Gd-DTPA leakage in CREAE may be predominantly by active vesicular or microvesicular transport rather than the alternative route for leakage of smaller molecules, from the opening of 'tight' junctions between endothelial cells, although this cannot be proved by histological methods (Bradbury, 1979). HRP is thought to be transported across the endothelial cell in vesicles and usually HRP is not seen as a continuous track between endothelial cells in CREAE (Kristensson and Wiśniewski, 1977; Kitz *et al.*, 1984). Our studies with the smaller molecular weight markers lanthanum and gadolinium nitrate also suggest the possibility of leakage by vesicular transport: we observed an increased number of endothelial vesicles containing lanthanide and the appearance of closed interendothelial junctions. Lack of evidence for opening of 'tight' junctions, however, does not exclude this possibility as the leaky junctions may have been missed during serial sectioning. Gd-enhancement in MS may also be due to increased vesicular transport since large numbers of pinocytic vesicles have been observed within the endothelia of cerebral venules in acute and subacute plaques (Brown, 1978).

Studies comparing Gd-albumin with Gd-DTPA enhancement showed that in some lesions there was more leakage of the smaller molecular weight compound Gd-DTPA (MW 550 Da) than Gd-albumin (MW 60 kDa). Leakage of protein would be expected in the initial inflammatory stages of the CREAE lesion, when it is thought that active demyelination is occurring, be it antibody or cell-mediated (Schluesener *et al.*, 1987; Linington *et al.*, 1988).

Immunoglobulin attached to the gadolinium complex led to enhancement of some lesions which were not seen with Gd-albumin alone. This observation warrants further investigation; it could be the result of selective transport or tissue binding of immunoglobulin, the latter because immunoglobulin is a basic protein and may be expected to bind nonspecifically to collagen and membranes in the extracellular matrix.

The histological similarities between CREAE and MS have been emphasized by Wiśniewski (Wiśniewski and Keith, 1977) and Lassmann (1983). Our study provides evidence that the functional changes in the blood-brain barrier are also similar. Enhancement is a feature of new lesions in both conditions and is detectable as early as lesions can be detected on unenhanced scans in MS and even earlier in EAE. The duration of enhancement is broadly similar (Miller *et al.*, 1988). The passage of HRP and Gd-protein through the blood-brain barrier in EAE provides evidence for a rather general increase in permeability of the blood-brain barrier to large as well as small molecules. It seems likely that the same is true in MS. There is evidence for persistent oedema in some chronic lesions in MS (D. Barnes, unpublished results). Whether this oedema reflects differential repair of the blood-brain barrier to larger molecules permitting passage of water into an extracellular space expanded as a result of myelin loss and not yet filled by astrocytic processes, or new inflammation with a generalized increase in permeability, is uncertain. It will be interesting to determine whether such differential repair takes place in CREAE. Since in CREAE Gd-enhancement was not seen without histological evidence of inflammation it seems safe to conclude that the observation of Gd-DTPA enhancement in MS also indicates the presence of inflammation and therefore of disease activity. The potential value of Gd-DTPA in monitoring treatment is obvious.

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A GENETIC STUDY OF IDIOPATHIC TORSION DYSTONIA IN THE UNITED KINGDOM

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SUMMARY

The inheritance of idiopathic torsion dystonia (ITD) was investigated in 100 British families containing 107 index cases with generalized, multifocal or segmental dystonia, and 79 secondary cases. Fifty-eight index cases had affected relatives, usually in two or more generations. Nearly half of the secondary cases were asymptomatic. Paternal age was increased among the 49 single cases, parental consanguinity was not increased and there was no evidence of genetic heterogeneity. Eleven cases (10.3%) were Jewish, which exceeded the number expected, but they did not differ clinically or genetically from non-Jewish cases The most likely explanation for the excess of Jewish cases is a founder effect in Eastern Europe.

We conclude that, in the UK, approximately 85% of cases of ITD are due to an autosomal dominant gene with about 40% penetrance and highly variable expression, possibly reflecting environmental influences. Approximately 14% of these inherited cases may represent new mutations. The remaining 15% are probably nongenetic phenocopies, but are not clinically distinguishable. There was no evidence in this study for the existence of autosomal recessive or X-linked forms of ITD. The estimated recurrence risk for first degree relatives of familial cases is 21%; the risk is lower for single cases. Of affected individuals, 75% will have developed symptoms or signs of dystonia by the age of 30 yrs.

INTRODUCTION

Idiopathic (or primary) torsion dystonia (ITD) is a disorder characterized by involuntary sustained muscle contractions, frequently causing twisting and repetitive movements or abnormal postures (Fahn *et al.*, 1987) without other associated neurological dysfunction such as pyramidal, cerebellar, sensory or intellectual deficits. It should be distinguished from symptomatic dystonia secondary to causes such as birth injury, degenerative extrapyramidal disorders, Wilson's disease, encephalitis or neuroleptic drugs (Marsden and Harrison, 1974; Marsden, 1988). The prevalence of ITD is unknown, but 330 per million has been suggested (Nutt *et al.*, 1988). As approximately three-quarters of all dystonia is idiopathic (Marsden, 1988), this is not a rare disease.

The clinical expression of this disease is highly variable and is principally determined by age of onset (Marsden and Harrison, 1974). Generalized ITD usually starts before the age of 20 yrs, often affecting the legs first. In the past, this form was called dystonia musculorum deformans; it is often familial (Zeman *et al.*, 1959) and can be very severe. In contrast, other familial cases have a later onset and present with segmental or focal

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dystonia, often affecting axial or cranial muscles; such cases can be mild or even asymptomatic (Zeman *et al.*, 1960). Postural tremor is also observed in dystonic patients and their relatives (Yanagisawa *et al.*, 1972; Marsden and Harrison, 1974; Couch, 1976); it is likely that this is sometimes the only manifestation of the condition (Bundey *et al.*, 1975; Cohen *et al.*, 1987; Forsgren *et al.*, 1988). So far, no consistent structural abnormality of the brain has been demonstrated in ITD (Zeman, 1970, 1976), but biochemical dysfunction in the basal ganglia or brainstem is suspected (Hornykiewicz *et al.*, 1986; Zweig *et al.*, 1988).

Although the aetiology of ITD is not known, it is clear that there is a genetic component. The exact nature of this has been controversial for several decades. Schwalbe described in 1908 a Jewish Lithuanian family containing 3 sibs with severe involuntary movements. It subsequently became clear that inheritance in this kindred was autosomal dominant with variable expression (Truong and Fahn, 1988). Among other early reports, some suggested recessive and others dominant inheritance. There was also a suggestion that ITD was common and more severe in Jews (Zador, 1936). Zeman and colleagues (Zeman *et al.*, 1959; Zeman and Dyken, 1968) concluded that inheritance was autosomal dominant with reduced penetrance, and that expression was often mild, including focal dystonia and tremor. These authors also suggested that isolated cases were either due to mutation or that they had relatives with mild undetected disease. The evidence for an autosomal recessive type was considered poor. Other studies (Johnson *et al.*, 1962; Larsson and Sjögren, 1966) supported these conclusions. An increased prevalence in Ashkenazi Jews was confirmed but not explained.

Eldridge (1970), on the basis of a family study in the USA, later proposed that there is a recessive form of ITD, particularly among Jews. Segregation analysis among sibs (assuming truncate ascertainment) was compatible with this mode of inheritance, and Jewish patients were said to have more severe disease, implying a recessive variant. A gene frequency of 1/130 for the Ashkenazi population and heterozygote advantage due to superior intelligence were proposed. Affected parents or offspring in Jewish families were explained by pseudodominance (the result of homozygote–heterozygote mating). Non-Jewish patients were thought usually to show dominant inheritance with 76% penetrance and more variable clinical features. Eldridge later proposed that all Jewish cases should be assumed to be recessive unless pseudodominance was excluded by 3 affected generations (Eldridge and Gottlieb, 1976).

In a previous study of 32 families in the UK, only 2 of which were Jewish, Bundey *et al.* (1975) also concluded that there were both autosomal dominant and recessive forms of ITD. Of 17 cases with generalized dystonia and childhood onset, 2 had an affected sib. There was no parental consanguinity, fitness was reduced and paternal age was increased among single cases, suggesting new mutation. A third to a half of these cases were thought to be recessive and the remainder mostly new dominant mutations. One of the cases with adult onset dystonia had an affected grandmother, but the majority of these cases were thought to be nongenetic phenocopies.

Studies in Israel (Korczyn *et al.*, 1980, 1981; Zilber *et al.*, 1984) have not supported the hypothesis that ITD amongst Jews is nearly always caused by an autosomal recessive gene. All familial cases had affected relatives in other generations; too many parents, cousins, uncles and aunts were affected; there were no affected sib pairs without other affected relatives, and parental consanguinity was not increased. A multifactorial model was not excluded but inheritance appeared dominant, with 51–68% penetrance and numerous 'formes fruste' including tremor and focal dystonia, as previously proposed for non-Jews. Paternal age was again increased among single cases. The increased prevalence among Ashkenazi Jews was difficult to explain, but nearly all traced their ancestors to Poland and Byelorussia, suggesting a founder effect.

It was noted that Eldridge had failed to show increased parental consanguinity in his cases, that the segregation ratio among sibs was incorrect because ascertainment was biased in favour of familial cases, and that the number of affected parents and children could not be explained by pseudodominance given the calculated gene frequency. Heterozygote advantage due to increased intelligence (Riklan *et al.*, 1976; Eldridge and Koerber, 1979) was also questioned.

Recently, further doubt has been cast on the concept of separate autosomal recessive and dominant varieties of ITD. The clinical course appears equally severe in non-Jews (Burke *et al.*, 1986) and it is now clear that age of onset rather than ethnic origin determines this. Moreover, preliminary results of a family study of early onset Jewish dystonia in the USA (Bressman *et al.*, 1988) also indicate dominant inheritance, with reduced penetrance and variable expression. A recessive form of ITD among Spanish gypsies has been proposed (Giménez-Roldán *et al.*, 1988) on the basis of 9 cases in 4 families with affected sibs and a high rate of parental consanguinity. This is questionable as it is not clear how many of the parents or offspring were examined and the families were from a population with a long tradition of consanguinous marriage.

The continuing controversy makes it difficult to give confident genetic advice to patients and relatives. Recent studies have concentrated on Jewish populations in the USA and Israel; accordingly, we undertook a genetic study of ITD in the UK with the aim of clarifying the inheritance of this condition and obtaining more accurate data for use in genetic counselling.

PATIENTS AND METHODS

Ascertainment

Criteria for index cases were (1) generalized, multifocal or segmental dystonia as defined by published criteria (Fahn *et al.*, 1987); (2) no pyramidal, cerebellar, sensory or intellectual deficit; (3) no history of perinatal asphyxia or kernicterus; (4) no other predisposing cause for dystonia; (5) no cause disclosed by investigation, particularly CT scan and biochemical tests; and (6) a clinical course compatible with ITD with no features to suggest secondary dystonia (Marsden, 1988) or other dystonic states such as DOPA-responsive dystonia, paroxysmal dystonias or alcohol-responsive myoclonic dystonia (Fahn *et al.*, 1987). Cases seen since 1959 were ascertained from the diagnostic index of the movement disorder clinic at the Institute of Psychiatry and King's College Hospital in London and the medical record departments of the National Hospitals for Nervous Diseases and the Hospital for Sick Children, London. In addition, consultant neurologists throughout the UK were asked for information about cases of idiopathic dystonia under their care. To avoid ascertainment bias, cases referred specifically because of known hereditary dystonia were not included. When a family was ascertained via more than one individual, each was designated as an index case.

A total of 289 index cases was initially ascertained (fig. 1); the available hospital notes of these cases were examined and those initially meeting the inclusion criteria were contacted by post with the permission of the relevant general practitioners and consultant neurologists; 160 cases were not seen for the reasons shown in fig. 1; 129 index cases from 125 families were seen. Three of these each had an affected relative who was independently ascertained but could not be seen; these relatives were also designated as index cases, giving a total of 132.

Using a standardized written protocol, comprehensive past medical and family histories were obtained and full neurological examinations performed by N.A.F. Severity and diagnosis were reassessed and subjects were recorded on videotape. Severity was classified by extent and quantified on a dystonia rating scale (Burke *et al.*, 1985). A total of 306 relatives was also interviewed and examined. Those definitely or possibly affected were recorded on videotapes which were subsequently reviewed independently by C.D.M. and A.E.H. for a final decision. Examined relatives were classified as affected if all 3 authors agreed that there was evidence of dystonia of any severity or if there was abnormal postural tremor. Relatives with

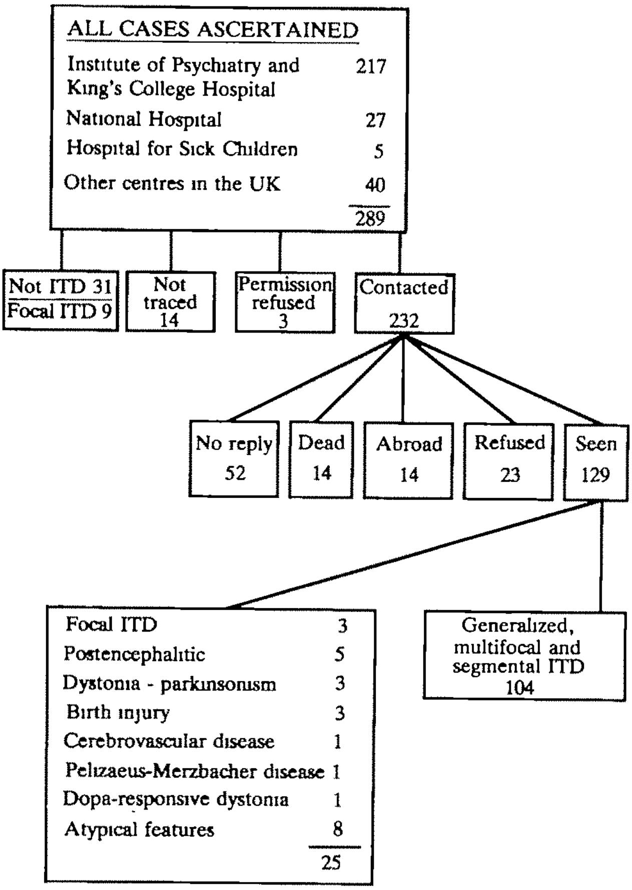
ALL CASES ASCERTAINED
Institute of Psychiatry and King's College Hospital 217
National Hospital 27
Hospital for Sick Children 5
Other centres in the UK 40
289
Not ITD 31
Focal ITD 9
Not traced 14
Permission refused 3
Contacted 232
No reply 52
Dead 14
Abroad 14
Refused 23
Seen 129
Focal ITD 3
Postencephalitic 5
Dystonia - parkinsonism 3
Birth injury 3
Cerebrovascular disease 1
Pelizaeus-Merzbacher disease 1
Dopa-responsive dystonia 1
Atypical features 8
25
Generalized, multifocal and segmental ITD 104

FIG. 1. Ascertainment sources and inclusion of index cases. Three of the index cases refused to take part in the study but were relatives of other index cases who were seen

only pronation of one or both wrists or asymmetric wrist flexion/finger extension postures with the arms outstretched were not considered as affected, as these were observed in 7 of 30 asymptomatic relatives of patients with neurological disorders other than movement disorders (N. A. Fletcher, A. E. Harding and C. D. Marsden, unpublished observations). Those not seen were defined as affected only if there was a clear history of dystonia or tremor, with confirmation by other relatives, family photographs, hospital records or videotapes wherever possible. In each of these cases, an assessment was made of the likely severity and extent of any dystonia.

On review, 25 index cases were excluded: dystonia was focal in 3, parkinsonism had developed in 3; a thorough review of the history was suggestive of an encephalitic illness in five, although the exact nature of this was unclear; evidence of perinatal birth injury with developmental delay emerged in 3, and these were probably examples of delayed onset dystonia; 1 had extensive cerebrovascular disease; 1 had DOPA-responsive dystonia; 1 had Pelizaeus-Merzbacher disease; 8 had atypical features such as intellectual impairment, deafness, dysmorphism, early development of severe dysarthria or fixed dystonic postures or the rapid development of generalized dystonia in adult life. In the latter cases, a secondary dystonia was strongly suspected but the nature of this could not be identified. A total of 107 index cases (of whom 104 were seen) from 100 families met the inclusion criteria and 260 of their relatives were examined. Altogether, 229 of 389 living first degree relatives of these index cases (49% of parents, 33% of sibs and 63% of children) were examined.

Methods of analysis and demographic sources

For purposes of segregation analysis, multiple incomplete ascertainment was assumed. Correction for bias of ascertainment was made by using Weinberg's proband method for calculation of segregation ratios among sibs. Ten sibs and 1 child who died before the age of 5 yrs, along with 10 children who were still below this age, were not included in the calculations. To allow for the late presentation of some cases, segregation ratios for sibs and children were also analysed after discounting those still under the age of 20 yrs and any who had died before this age.

Segregation ratios (as shown in Table 2) were calculated both for relatives seen personally and for all relatives. Confidence limits of segregation ratios were based on all relatives, including those not examined. The lower limit assumes that none of those not examined and said to be normal was affected; the upper limit assumes that among relatives who were not examined, there was the same proportion of asymptomatic affected individuals as seen in examined relatives

Population structure, mean family size and paternal age data for the UK were obtained from the Office of Population Censuses and Surveys (1984, 1987). Mean paternal ages before 1961 are not available and were estimated from known (1938–1960) or estimated (pre-1938) annual mean maternal ages and mean husband-wife age difference as described by Bundey *et al.* (1975).

Mutation rate was calculated by Haldane's indirect method (Vogel and Motulsky, 1986). Fitness of index cases was estimated by comparison with the general population. Population mean family sizes were available from 1920 onward and we assumed that only cases aged over 35 had completed their families. The prevalence of generalized, multifocal and segmental ITD was estimated to be 100/million based on a recent estimate of 330/million for all forms of idiopathic dystonia (Nutt *et al.*, 1988); approximately one-third of the cases ascertained by these authors had dystonia which was generalized or affected more than one body part on the basis of the clinical data given. Demographic data for the Jewish community in the UK were kindly supplied by the Community Research Unit of the Board of Deputies of British Jews.

Data for age of onset, severity rating and number of children were not normally distributed, comparisons using these data were by χ^2 tests with Yates' correction. The number of Jewish cases was small and comparisons with non-Jewish cases were made using two-tailed Fisher's exact tests. Normally distributed data such as paternal age were analysed by Student's t tests. Calculation of intrafamilial correlation for age of onset and severity score were by Kendall's rank correlation test (coefficient = τ) Analysis of variance was by a Kruskal-Wallis (nonparametric) test.

RESULTS

Index cases

There were 45 male and 62 female index cases; the sex difference was not significant ($\chi^2 = 2.39$, $P > 0.1$). The geographical distribution of cases was as shown in fig. 2. Dystonia was generalized in 53 (49.6%), multifocal in 7 (6.5%), segmental in 46 (43.0%) and 1 (0.9%) had hemidystonia. All multifocal cases had widespread dystonia involving the lower limbs; their ages of onset ranged from 4 to 23 (median 8) years and all but one started before 20 yrs. They were therefore considered along with the generalized cases. Males and females did not differ with respect to the numbers with generalized/multifocal and segmental dystonia ($\chi^2 = 0.01$, $P > 0.8$).

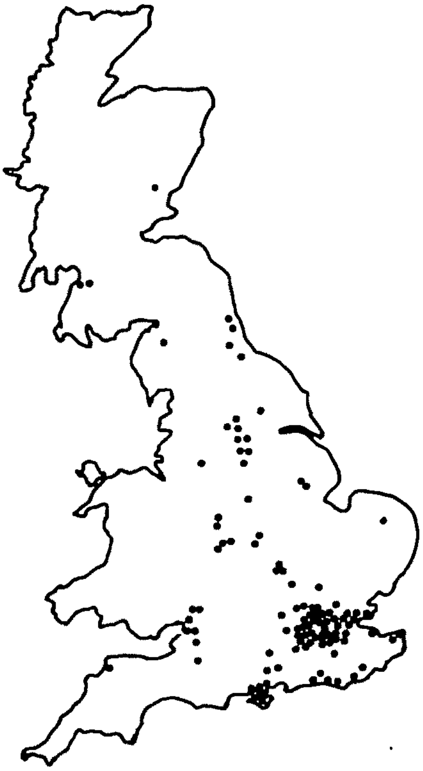

FIG 2 Geographical distribution of 107 index cases with ITD

Age of onset (known in 106 cases) was from 2 to 70 (median 13) years; 65% had developed symptoms by the age of 20 (87% of generalized and 37% of segmental cases). Age of onset (fig. 3A) for generalized and multifocal cases was from 2 to 48 (median 9) yrs and for segmental cases 5 to 70 (median 35) yrs ($\chi^2 = 24.9$, $P < 0.001$).

Eleven index cases (10.3%, standard error (SE) 2.9) from 9 families were Jewish

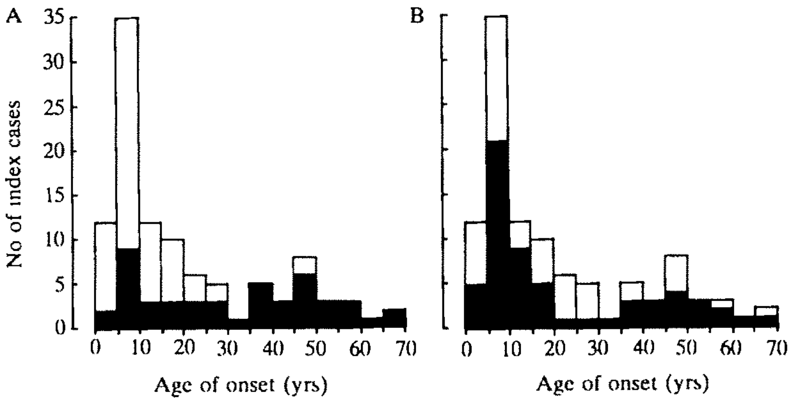

FIG. 3 The distribution of ages of onset for 106 index cases with ITD is shown in A and B. In A, the shaded area represents cases with segmental dystonia. In B, the shaded area represents familial cases. Age of onset was not known for 1 index case.

and this proportion was higher than expected; the UK has a Jewish population of 330,000 or 0.6% ($P < 0.001$). Among 30 cases from London, 6 were Jewish (20%), in contrast to the 3% of the London population overall. Nine Jewish cases had ancestors from Poland, Russia or Lithuania.

Affected relatives

Among 260 examined relatives, there were 37 (14%) secondary cases; 18 (48.6%) were asymptomatic. Another 42 relatives, either dead or not available for examination, were affected on the basis of history. There were therefore 79 secondary cases overall and their clinical features are shown in Table 1. Ages of onset were known for 22 secondary cases (fig. 4) and were slightly later than for index cases (2–75 yrs, median 18.5).

TABLE 1 CLINICAL FEATURES OF 79 SECONDARY CASES

	No	*(%)*	*Examined*
Generalized dystonia	15	19.0	6
Multifocal dystonia	6	7.6	2
Segmental dystonia	25	31.6	13
Focal dystonia	27	34.2	11
Postural tremor	6	7.6	5

Pedigree and segregation analysis

Fifty-eight of the 107 index cases had affected relatives and 49 were single cases. Fifty-one index cases from 45 families had affected relatives in more than one generation (multigeneration cases); members of three generations were affected in 16 families and

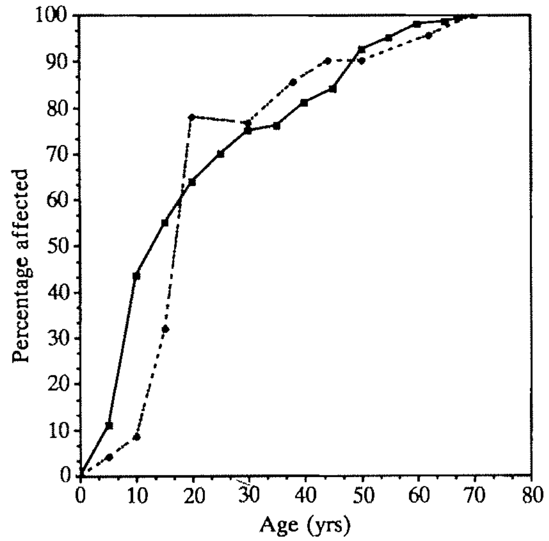

FIG 4. Cumulative age of onset plots for cases of ITD Continuous line = index cases; broken line = secondary cases

four in another. Seven index cases from six families had affected relatives in only one generation; 5 of these had affected sibs and 2 each had an affected sib and first cousin. Sixteen unaffected relatives, of whom 8 were examined, had both descendants and ancestors or sibs affected. The 100 families contained 33 parents who had affected sibs or antecedents as well as affected descendants; 14 of these (42.4%) were affected. One instance of parental consanguinity was observed (first cousin parents of a single case).

TABLE 2 SEGREGATION ANALYSES

	Affected/total			
	Examined relatives	*All relatives*	*Ratio*	*Confidence limits*
All cases				
Parents	27/103	33/214	0.154	0.154–0.182
Sibs	13/70	22/210	0.105	0.105–0.148
Sibs >20 yrs	11/53	16/174	0.092	0.092–0.121
Children	5/49	9/81	0.111	0.111–0.160
Children >20 yrs	2/26	5/49	0.102	0.102–0.163
Single generation cases				
Sibs	4/31	9/120	0.075	0.075–0.117
Sibs >20 yrs	1/25	6/106	0.057	0.057–0.104

All calculations are corrected for relatives less than 5 yrs old or who died before this age. Ratios and confidence limits are based on all relatives, including those not examined The lower limits assume no additional asymptomatic cases among those not examined; the upper limits assume that this group contained equal proportions of symptomatic and asymptomatic affected individuals, as was observed in examined relatives.

The 107 index cases had 64 affected first degree relatives (Table 2). There were no significant differences between the proportions of parents, sibs and children affected, and in particular no excess of affected sibs was observed. An additional analysis of sibs and children over the age of 20 yrs was made to allow for those younger than this who may yet develop symptoms, but the proportions affected were similar. Among isolated cases or those with only affected sibs (single generation cases), the confidence limits of the segregation ratio for affected sibs (Table 2) did not accommodate that of 0.25 expected for autosomal recessive inheritance. Among the 58 familial index cases, 46 had at least 1 affected ancestor and the proportion of affected sibs in this group was 12 out of 76 (15.8%); for sibs aged over 20 yrs, the proportion was 9 out of 54 (16.7%). The number of familial cases with either an affected ancestor or sib was 54. These had 39 children of whom 5 (12.8%) were affected; if children under 20 are discounted, 2 out of 20 (10%) were affected.

Tests for genetic heterogeneity

The ages of onset for familial cases (range 4–67, median 12 yrs) and for single cases (range 2–70, median 17 yrs) were similar ($\chi^2 = 3.51$, $P > 0.25$) and are shown in fig. 3B. Familial and nonfamilial groups contained similar proportions of generalized or multifocal cases (34/58 and 27/49, respectively, $\chi^2 = 0.03$, $P > 0.5$). Although the distribution of age of onset in index cases suggests bimodality, the mode at 45–50 years is not significant ($P > 0.10$).

The clinical features of single generation and multigeneration cases were compared and there were no significant differences (Table 3). In particular, as was observed when comparing familial and nonfamilial cases, ages of onset were similar in the two groups. Analysis of extent of dystonia in secondary cases of generalized/multifocal and segmental index cases (Table 4) did not suggest that these clinical types are genetically distinct. Comparison of Jewish with non-Jewish cases (Table 5) revealed no differences in extent of dystonia or numbers of single generation and multi-generation cases. Analysis of variance (Kruskal-Wallis test) detected no evidence of heterogeneity using ages of onset (H = 26.61, $P > 0.25$) or severity ratings (H = 17.0, $P > 0.95$).

TABLE 3. CLINICAL FEATURES OF MULTIGENERATION AND SINGLE GENERATION INDEX CASES

	Multi-generation cases (51)	*Single generation cases (56)*	
Leg onset	23	15	$P > 0.05$**
Limb onset	33	35	$P > 0.9$**
Generalized†	31	30	$P > 0.5$**
Segmental	20	26	
Jewish	6	5	$P = 0.36$*
Good response to Benzhexol	10	6	$P = 0.20$*

*Fisher's exact test, ** χ^2 test. † Includes multifocal cases

TABLE 4 DISTRIBUTION OF GENERALIZED AND SEGMENTAL DYSTONIA WITHIN FAMILIES CONTAINING MORE THAN ONE CASE

	Secondary cases	
	Generalized/ multifocal	*Segmental*
Index cases		
Generalized/multifocal	14	29
Segmental	7	23

$\chi^2 = 0.35$, $P > 0.05$ (6 secondary cases with postural tremor not included)

TABLE 5. CLINICAL AND GENETIC FEATURES OF DYSTONIA IN JEWISH AND NON-JEWISH CASES

	Jewish	*Non-Jewish*	
Multigeneration	6	45	$P = 0.36$
Single generation	5	51	
Generalized/multifocal	7	53	$P = 0.87$
Segmental	4	42	

Paternal age, fitness and mutation rate

Among familial cases, mean paternal age was 30.13 yrs; a mean of 29.3 yrs would be expected based upon mean paternal ages in the population for the same years of birth. Comparison between actual and population mean paternal ages for familial cases (paired t test) showed no significant difference ($t = 0.69$, $P > 0.4$). Among single cases, mean paternal age was 32.8 yrs with an expected value of 29.9 and was significantly increased ($t = 2.19$, $P < 0.05$).

Fifty-one index cases born between 1920 and 1952 had 71 (mean 1.39) children. Population mean family size for this period was 2.12, giving an approximate fitness for affected individuals (f′) of 0.66 (1.39/2.12). Assuming the prevalence of generalized and segmental ITD (I) to be 100/million (Nutt *et al.*, 1988), at genetic equilibrium the mutation rate (μ) is given by: $2\mu = (1\text{-}f')I$ (Emery, 1986) $= 17\times10-6$ gametes.

Index cases originally reported by Bundey et al. (1975)

All the patients reported by Bundey *et al.* (1975) were also included in this study due to the use of the same ascertainment sources. Of the 17 cases of juvenile onset generalized ITD who were thought by Bundey *et al.* to represent autosomal recessive inheritance or new dominant mutations, 10 were either dead, not traced or refused to be seen. Of the 7 cases seen, 4 have had 7 children. Of these, 5 were examined and are normal but are less than 5 yrs old; another 2 aged 13 and 15 yrs are said to be normal but permission to examine them was refused. However, 3 of the 7 index cases each has a parent who has developed dystonia. One parent developed writer's cramp and oromandibular dystonia in adult life but refused to be seen in the earlier study. The other 2 are asymptomatic and were normal when examined previously; 1 now has torticollis and the other segmental dystonia and postural tremor. Another case who refused to be seen for the present study had an affected cousin who was independently ascertained and did participate. The mother of this individual was found to have mild action dystonia of one hand. Thus 4 cases appeared to have inherited ITD from a parent, 3 of whom were mildly affected much later than their severely affected offspring and 1 of whom, although not examined, is apparently still normal.

Bundey *et al.* (1975) also reported 12 cases with later onset, nongeneralized dystonia, who were considered to be mostly nongenetic phenocopies. Seven of these are now dead, or refused to be seen, including 1 case who was said to have had an affected grandmother. Five were seen and all remain isolated cases; only 2 have had children and all 5 of these are aged over 20 yrs and are normal on examination.

Tests for polygenic inheritance

The maximum frequency in first degree relatives predicted by this model is the square root of that in the population (Emery, 1986). Even assuming that all forms of idiopathic dystonia (330 per million; Nutt *et al.*, 1988) are inherited, the maximum figure predicted is 1.8%, whereas the lowest observed was 10.5% among sibs ($P < 0.001$). A polygenic rather than a single gene model is preferable when the proportion of affected sibs is more than 2.5 times greater for those cases with an affected parent than for those without (Vogel and Motulsky, 1986). Among sibs of 33 cases with an affected parent and 74 without, 14.6% and 9.0%, respectively, were affected (ratio = 1.62).

There were 62 female and 45 male index cases; given polygenic inheritance, it might be expected that males, the less frequently affected sex, would have more affected first degree relatives (Emery, 1986). This was not observed (males 12.2% of first degree relatives affected, females 12.6%).

Intrafamilial correlation

Many secondary cases were asymptomatic and in only 28 was the age of onset known. Correlation of onset ages between index cases and affected relatives using rank correlation ($\tau = 0.085$, $P > 0.5$) was low. The same applied to severity score ($\tau = -0.054$, $P > 0.5$).

DISCUSSION

Two features of ITD have complicated studies of its genetic basis. First, the highly variable expression has hindered the detection of affected relatives (Zeman *et al.*, 1959) and there has been uncertainty about the significance of mild manifestations. Moreover, although familial ITD may develop late in life and be segmental or focal, some genetic studies have excluded older or nongeneralized cases (Zilber *et al.*, 1984; Bressman *et al.*, 1988). Secondly, diagnostic criteria have varied and cases of secondary dystonia have been reported as inherited ITD (Oswald *et al.*, 1986). We have tried to minimize these problems by including childhood, adolescent and adult onset cases, whether generalized, multifocal or segmental, and using rigorous diagnostic criteria for ITD. Therefore our sample contained approximately equal numbers of generalized and segmental cases, with a wide range of ages of onset, in whom the diagnosis was as secure as possible. It has been suggested that because age of onset for all forms of ITD is bimodally distributed, mainly due to differences between generalized and focal cases, later onset cases may represent a separate nongenetic population (Bressman *et al.*, 1988). We also found that severity was related to age of onset, with generalized cases almost all developing symptoms before 20 years, but segmental ITD developed at all ages rather than forming a distinct later onset group. Moreover, familial and nonfamilial cases did not differ in terms of age of onset or severity of dystonia. In generalized, multifocal and segmental ITD, hereditary cases cannot be identified by age of onset or severity.

If generalized, segmental and focal dystonia are related, albeit in a poorly understood manner, our exclusion of focal cases is admittedly arbitrary. This was principally due to concern about heterogeneity; the relationship of focal to generalized dystonia is less clear than that of segmental dystonia and there is therefore more uncertainty about shared aetiology (Marsden, 1976; Fahn *et al.*, 1987).

Although the concentration of our index cases in south-east England is striking and reflects our ascertainment sources, the sample was probably representative of dystonia throughout the UK. Patients had been referred from most areas of the country and the distribution is similar to that of the population, over 25% of which live in London and the surrounding counties (Office of Population Censuses and Surveys, 1984). There is no reason to suggest that secondary or tertiary referral patterns introduced bias into the genetic anlayses; this was excluded as much as possible in the definition of index cases.

Almost a half of relatives found to be affected were asymptomatic, but overall 58% were as severe as (or worse than) the index cases. The spectrum of dystonia in secondary cases was very wide, ranging from generalized to focal, and 6 secondary cases had only postural tremor. It may be questioned whether such individuals are affected, but there are several reasons for concluding this. First, tremor is seen frequently in dystonic patients (Zeman *et al.*, 1959; Marsden and Harrison, 1974; Couch, 1976, Sheehy and Marsden, 1982). Secondly, it has been detected in relatives and probands in almost every genetic study undertaken (Eldridge, 1970; Bundey *et al.*, 1975; Zilber *et al.*, 1984) and such a frequent coincidence with essential tremor is unlikely. Thirdly, there is evidence of a shared pathophysiology between some cases of essential tremor and dystonia (Rosenbaum and Jankovic, 1988), and some patients with essential tremor have signs of dystonia (Critchley, 1949; Baxter and Lal, 1979). Finally, patients with essential tremor may be more likely to develop dystonia in response to trauma (Jankovic and Van Der Linden, 1988). Therefore tremor and dystonia seem to be associated (Lee, 1987). These 6 relatives are thus almost certainly affected. This conclusion affects the final results only minimally.

The finding of approximately equal proportions of affected parents, sibs and children is evidence against the existence of a large group of autosomal recessive cases, as this would produce an excess of affected sibs. Fifty-one cases had affected relatives in two or more generations. In these the data are most consistent with autosomal dominant inheritance and reduced penetrance. If this is the case there were 16 normal obligate gene carriers.

A crucial question concerns inheritance among the 56 single generation index cases, of which 7 had affected sibs and 49 were isolated. One possibility, based on the proportion of affected sibs (8%), is that inheritance is autosomal recessive in approximately one-third. Against this hypothesis, implying a rare recessive disorder, is the low incidence of parental consanguinity in this (1 case) and other studies (Eldridge, 1970; Zilber *et al.*, 1984). In addition, we found no statistical or other evidence of clinical or genetic heterogeneity to support separate dominant and recessive forms. Negligible intrafamilial correlations for age of onset and severity suggest the interaction of a single gene with environmental effects rather than genetic heterogeneity (Haldane, 1941). Jewish cases, suggested to show recessive inheritance (Eldridge, 1970), did not differ from non-Jewish ones, either clinically or in terms of incidence of affected relatives in other generations. A separate autosomal recessive form of ITD is therefore unlikely. A dominant gene of reduced penetrance may sometimes give rise to only affected sibs without necessarily implying a separate recessive variant (Opitz, 1981).

Polygenic inheritance, from which a major gene of reduced effect needs to be distinguished (Vogel and Motulsky, 1986), is unlikely; too many affected relatives were observed, even assuming the highest possible frequency for hereditary ITD. Moreover, no other predictions based on a polygenic model agreed with the data. There was no evidence of an X-linked form of ITD in this series. There were more female than male cases, and no difference in severity between the sexes.

In the light of these findings we suspect that, as proposed 30 years ago (Zeman *et al.*, 1959), most single cases have dominant ITD but are either new mutations or have inherited the disease from a phenotypically normal carrier parent. Significantly increased paternal age, observed in single cases in this and other studies, suggests the existence of new mutations (Penrose, 1955).

Although the inheritance of ITD appears to be largely autosomal dominant with reduced penetrance, the overall segregation analysis cannot reliably be used to calculate penetrance because it cannot be assumed that all 107 index cases were genetic. However, if autosomal recessive inheritance is discounted, parents with more than one affected child, or with one child and an affected ancestor or sib are almost certainly gene carriers; there were 33 such parents, of whom only 14 (42.4%) were affected. Fifty-four cases with either a sib or an ancestor affected, that is familial cases irrespective of affected children, had 39 children of whom 5 (12.8%) were affected; this suggests 25.6% penetrance. Similarly, 46 cases had an affected ancestor and so were familial, irrespective of affected sibs, and were not new mutations; 15.8% of their sibs were affected, giving a penetrance of 31.6%. Among familial cases overall, the figures for penetrance in sibs and children were therefore lower than the 42.4% observed in parents. This is partly because segregation ratios among sibs and children will err on the low side if some relatives become affected quite late, as we observed among the parents of cases previously studied by Bundey *et al.* (1975). Theoretically, analyses of sibs and children aged over 35 yrs, by which age 80% of our cases were apparent, would have been more informative but in practice the numbers were too small to be of value. Separate analyses were therefore made of those over 20 yrs but this made little difference in practice and the numbers were still small. Also, the true proportion of affected sibs may be higher because fewer could be examined. The most reliable estimate of penetrance is therefore that among parents.

Central to the controversy surrounding the inheritance of ITD has been the unexplained excess of Jewish cases. We found a much higher than expected number of Ashkenazi Jews among our cases. Over 60% of the Jewish community is centred in London where our index cases were also concentrated, but this does not adequately explain the excess of Jewish cases which was also found within the London area. The notion of a recessive ITD variant arose mainly to explain this consistent observation (Eldridge, 1970), but there is increasing evidence against recessive inheritance of ITD in Jews, both from our data and recent studies of large numbers of Jewish families (Zilber *et al.*, 1984; Bressman *et al.*, 1988). Increased mutation among Jews is unlikely. The mutation rate estimated from the present study (17×10^{-6} gametes) is very similar to 16×10^{-6} estimated in Israel (Zilber *et al.*, 1984) despite no reliable estimates of the frequency of ITD in the UK and the inclusion only of generalized cases in the Israeli estimate. Moreover, the proportion of single cases is no higher in Jewish patients (Zilber *et al.*, 1984). An alternative hypothesis is suggested by the frequency of ITD among Jews with ancestors from Poland, Byelorussia and the Baltic territories. In the light of this geographical concentration, previous genetic drift or a founder effect in a genetic isolate is perhaps the best explanation (Zilber *et al.*, 1984). It is also possible that the widely recognized association of ITD with the Ashkenazi Jewish population has led to earlier and more accurate diagnosis of the condition in this ethnic group.

If fitness in ITD (f′) is 66%, 34% of phenotypically expressed ITD genes are lost by selection in each generation and an equivalent number of new mutations maintain genetic equilibrium. However, not all mutations will be expressed because penetrance (P) is only 42%. The approximate proportion of ITD cases due to mutation is 1-f, where f is the fitness of all gene carriers (Emery, 1986). Assuming fitness of phenotypically normal carriers is 1.0, $f = 1-(1-f')P$, $= 1-(0.34\times0.42)$, $= 0.86$. Therefore approximately 14% (100−86) of inherited ITD cases should be due to new mutations and the total number of cases is presumably maintained by new cases inherited from phenotypically normal parents. If 14% of inherited cases are new mutations, the number

TABLE 6 OBSERVED AND THEORETICAL RECURRENCE RISKS IN ITD

	Observed	*Theoretical*
Familial case		
Child	12.8%	21%
Sib	15.8%	21%
Sib >20 yrs	16.5%	21%
Isolated case		
Child	–	14%
Sib	7.5%	8%
Sib >20 yrs	5.7%	8%

Calculated risks assume that 85% of cases of ITD are due to autosomal dominant inheritance with 42% penetrance, of which 14% are new dominant mutations. Observed risk to sibs of isolated cases calculated from proportion of affected sibs of all index cases without affected antecedents or children of affected parents (33) should equal (0.86)P multiplied by the overall number of genetic cases; this gives an estimate of 91 genetic cases out of 107 (85%). The other 16 may be nongenetic phenocopies. Of the 91 genetic cases, the estimated number due to new mutation is $0.14 \times 91 = 13$.

Observed and theoretical recurrence risks to relatives of patients with ITD are given in Table 6. These depend on whether the patient in question is a familial or an isolated case. If the former, the risk to sibs or offspring would be expected to be 21% (P/2) based on the penetrance observed among parents. In our 107 cases, we estimate that of the 49 isolated cases, 16 (33%) were nongenetic and 13 (26%) represented fresh mutations; a theoretical risk to offspring of single cases is therefore $0.67 \times P/2 = 14\%$. That to sibs is $0.41 \times P/2 = 8\%$. Our data suggest that the risk to relatives may be reduced the longer they remain unaffected, falling by 50, 75 and 90% at the ages of 15, 30 and 50 yrs respectively (fig. 4).

In conclusion, this study of a large cohort of patients in the UK suggests that the great majority of cases with generalized, multifocal and segmental ITD have a genetically determined disorder of autosomal dominant inheritance. The gene appears to be characterized by variable expression and reduced penetrance, such that a child or a sib of a familial case has a 21% risk of also being affected. For isolated cases, the risks are lower. These observations apply only to generalized, multifocal and segmental dystonia. The genetic contribution to adult onset focal dystonias remains to be established.

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SEMANTIC MEMORY LOSS IN DEMENTIA OF ALZHEIMER'S TYPE

WHAT DO VARIOUS MEASURES MEASURE?

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SUMMARY

This paper examines three methodological issues concerning the measurement of semantic memory impairment in brain-damaged patients. Ten carefully selected patients with dementia of Alzheimer's type (DAT) and anomia were studied. A battery of perceptual tests and direct tests of semantic memory led to the conclusion that these patients represented a homogeneous group having a prominent deterioration of their semantic memory store without visual perceptual deficits.

The first issue addressed in this patient group was whether verbal fluency impairment accurately reflected the loss of semantic memory. It was found that verbal fluency (generation of semantic category lists) was impaired due to two major constraints: deterioration of semantic memory store, and variable difficulties in semantic search. Verbal fluency, therefore, reflects semantic memory loss to some degree, but is not a direct test of semantic memory store in DAT. The second issue was whether semantic memory impairment in our patients conformed to the 'semantic storage disorder' syndrome hypothesized by Shallice (1987). It was shown that, consistent with this hypothesis, the patients demonstrated co-occurrence of consistency of errors, loss of semantic cueing, and preserved superordinate knowledge with loss of detailed knowledge of concept items. The third issue was whether semantic cueing and semantic priming are altered in a similar manner in DAT. It demonstrated that semantic cueing and semantic priming, using the same words whose concepts were degraded in semantic memory, yielded an entirely different pattern of results. Cueing and priming therefore may not be used interchangeably in the study of semantic loss after brain damage.

INTRODUCTION

Semantic memory contains the permanent representation of our knowledge of concepts, of words and their meanings, and in essence our knowledge of the world. Modern cognitive psychology considers semantic memory as a separate subcategory within long-term memory (Squire, 1987). Tulving (1972, 1983) distinguished it from episodic memory, that portion of long-term memory which is autobiographical, in that semantic memory includes shared cultural experience, is acquired early, is overlearned, and is resistant to degradation except in the most extreme circumstances. As a possible module within an information-processing framework, semantic memory would be involved in the cognitive processes by which we recognize objects and retrieve their labels, by which we gain access to the meaning of a word when we read it, and would also be involved

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in the mechanisms by which our knowledge of concepts is converted into words for speaking. All of these mental processes are crucially dependent on our retrieving stored information from semantic memory, automatically and within several hundred milliseconds.

Recent evidence from brain-damaged subjects suggests that impairments of semantic processing might be a critical factor in the occurrence of some well-known neurological syndromes, such as the language disturbance in dementia (Bayles and Tomoeda, 1983; Bayles and Kaszniak, 1987), certain forms of aphasia (Howard and Orchard-Lisle, 1984), deep dyslexia (Morton and Patterson, 1980), and visual associative agnosia (Warrington, 1975). There are methodological difficulties, however, in ascertaining the presence of a true disturbance within semantic memory as opposed to difficulties that prevent reliable access to the knowledge permanently represented within this functional system. Research on the nature of the comprehension deficit in aphasia has not yet successfully distinguished between these alternative possibilities (e.g. Zurif *et al.*, 1974; Whitehouse *et al.*, 1978; Gainotti, 1982, 1987; Butterworth *et al.*, 1984).

Studies of dementia of Alzheimer's type (DAT) provide more consistent evidence for a genuine semantic memory disorder. The anomia in DAT patients is often disproportionally severe in relation to other language deficits, it is an early manifestation, and it progresses with overall language deterioration (Appell *et al.*, 1982; Kertesz *et al.*, 1986). While severely demented patients may be anomic due to impaired visual perception (Lawson and Barker, 1968; Rochford, 1971; Kirshner *et al.*, 1984), patients with early or moderate DAT usually have relatively minor perceptual problems. Studies of the comprehension impairment for words and pictures observed in this population (reviewed in more detail later) have begun to support the hypothesis of a central disturbance in the representation of object concepts—the patients fail to categorize words and pictures because they no longer retain a fully specified description of their meaning (Warrington, 1975; Bayles, 1982; Bayles and Tomoeda, 1983; Martin and Fedio, 1983; Huff *et al.*, 1986).

In this paper, we examine a variety of possible approaches to measuring semantic impairment: item specificity, priming and cueing effects, and verbal fluency.

Item specificity

Patients with DAT are known to lose knowledge pertaining to specific words and pictures on probe questions. Huff *et al.* (1986) selected a group of DAT patients who showed no deficit on visual perceptual testing. These authors then measured the anomia of the patients on a battery of test pictures. They compared the anomia with the loss of word comprehension for the same exemplars and observed a definite item-to-item correspondence; objects that were not labelled by the patients were also not matched to the corresponding word in a forced-choice task comprising semantically confusable exemplars.

The presence of item specificity does not characterize the performance of all patients with impaired comprehension and anomia. Butterworth *et al.* (1984) demonstrated that aphasic patients do not show any correlation between the items whose line drawings they fail to name and errors made on a word-picture matching task. They concluded that the comprehension and naming deficit in aphasia reflects a disturbance in mapping word-level information to and from stored representations of their meaning.

The inconsistency between performance to the same item on different tests and on different occasions observed in aphasic patients must stand in marked contrast to this notable consistency suggested by Huff *et al.* (1986) in DAT patients. Warrington and Shallice (1979) have included the distinction as one amongst a number of criteria which they claim may be used to differentiate storage from access disorders of semantic knowledge. Failure to contact a stored representation reliably would yield a lack of consistency across tasks or sessions with respect to individual items. Alternatively, consistent failure on the same exemplars (and corresponding success on others) can be taken as evidence for actual loss of semantic representations, a loss which would affect knowledge of the same item when tested repeatedly. Other criteria which they claimed indicated the presence of a semantic storage disorder included the preferential loss of detailed knowledge of objects, with retention of superordinate knowledge, along with loss of 'priming effects'.

There have been criticisms of this analysis of consistency in brain-damaged patients (e.g., Humphreys *et al.*, 1988), based primarily on the claim that 'noisy' access procedures may affect connectivity to specific representations in semantic memory, namely those requiring a greater amount of perceptual information for identification. Shallice (1988*b*) has provided cogent arguments against this point of view, in particular noting that it could apply only to visual objects. The explanation is not valid for cases fitting the criteria of an access disorder in the verbal domain. We take as a reasonable point of departure, then, the claim that consistent failure on the same object concepts is the outcome of damage to their stored representation in semantic memory.

Priming and cueing effects

Impairments of semantic priming and semantic cueing are considered to be evidence of a semantic memory disturbance by a number of authors (Nebes *et al.*, 1984; Shallice, 1987). As Shallice (1988*a*) argued 'On the degraded store deficit, if an item cannot be identified, it should not be possible to prime it' (p. 282).

A test of this hypothesis is complicated by ambiguity in the notion of 'priming' used by Warrington and Shallice (1979). Although Shallice (1987) refers to priming in the context of on-line testing of speeded reaction times for performing word/nonword decisions, this technique was not in fact used in Warrington (1975) or Warrington and Shallice (1979). In those papers, the 'priming' consisted of verbal or pictorial cues offered to assist the exemplar in tasks such as object naming. Although Shallice would consider that on-line priming must give the same results in semantic storage disorders as the off-line cueing has produced, this basic assumption has never really been tested, much less established. Given the view expressed by Shallice (1988*a*) that priming (along with consistency) has the greatest face validity of all the proposed criteria for distinguishing storage from access disorders, the status of priming in patients with demonstrable loss of semantic descriptions is obviously in need of empirical investigation.

Verbal fluency

Verbal fluency is a clinical measure taken by many to be a diagnostic of a semantic memory disturbance (Rosen, 1980; Huff *et al.*, 1986; Ober *et al.*, 1986; Butters *et al.*, 1987; Flicker *et al.*, 1987). The two verbal fluency tasks used most frequently to investigate brain damage require the patient to produce words that begin with a specified letter (Thurstone and Thurstone, 1938) and words that are members of a more restricted category such as animals (Newcombe, 1969). Many researchers have documented decreased word fluency on both types of tasks in patents with probable DAT (Pearce and Miller, 1973; Miller and Hague, 1975; Rosen, 1980; Huff *et al.*, 1986; Ober *et al.*, 1986; Butters *et al.*, 1987; Flicker *et al.*, 1987).

The verbal fluency task is an indirect measure of semantic memory impairment because it is complex with many cognitive demands. Rosen (1980) suggested that the task involves both linguistic and fluency components. The linguistic component referred to the intactness of stored category items in semantic memory, and the patient's ability to access these items. Fluency referred to the speed and efficiency of the search procedure involved in retrieving items from this semantic store. Diesfeldt (1985) delineated several factors which might influence such search procedures. Impaired initiative and spontaneity, impaired ability to generate imagery, impaired working memory, attention deficits, and a high arousal level could all adversely affect semantic memory search. Impaired ability to break down categories into subcategories and to search them systematically would also impair verbal fluency, as would a general slowing of the search procedures. The existence of these multiple possibilities makes it difficult to interpret the impaired verbal fluency in dementia directly in terms of a straightforward breakdown of semantic memory.

Rosen (1980) considered that in early to moderate DAT, decreased verbal fluency was due to impaired search mechanisms, since the patients would offer exemplars of animal category members which were common and clear cases, but would not supply less common exemplars. In more advanced cases there was an additional breakdown within the semantic structure itself, impairing report of even these familiar exemplars. Miller and Hague (1975) noted that DAT patients with a marked reduction in verbal fluency on formal testing continued to use comparatively 'rare' words in free speech. This suggested to them that the problem was generally one of word retrieval rather than a loss of the word's representation in the lexicon.

Diesfeldt (1985) and Ober *et al.* (1986) concurred that impairment of semantic search procedures was the predominant cause of decreased verbal fluency early in dementia. Diesfeldt demonstrated that DAT patients had at least intact superordinate knowledge concerning items they had failed to generate on verbal fluency testing, and inferred that the difficulty lay more in item retrieval than in actual loss of the lexical item from semantic memory. Weingartner *et al.* (1981) and Ober *et al.* (1986) noted a correlation between impairment on semantic category fluency and other forms of cognitive impairment. Specifically, they speculated that the attentional deficits associated with dementia might play a major role in impairing verbal fluency.

Other studies, however, raised doubts about the claim that defective search and retrieval mechanisms are primarily responsible for impaired fluency on list generation tasks. Warrington (1975) noted that patients with dementia generally lose semantic knowledge at a more detailed level, while preserving knowledge at a more general, superordinate level. Thus the demonstration by Diesfeldt (1985) of preserved superordinate knowledge in his DAT patients does not indicate that their semantic memory store is intact. Butters *et al.* (1987) found that in their series of patients with milder DAT, there was impairment in producing animal names but not on letter fluency tasks. They suggested that this reflected the presence of early linguistic deterioration at the level of semantic memory causing a reduction in the number of category exemplars available to the patient. Such a deficit would appear most prominently in testing a limited abstract category such as animals. In contrast, additional impairments (i.e. in search procedures) would only occur with more severe forms of the illness. There appears to be disagreement, therefore, as to whether decreased verbal fluency alone can be taken as a measure of semantic memory impairment in dementia.

Purpose of investigation

In this paper, we examine three methodological issues related to semantic memory impairment in patients with DAT (1) the extent to which loss of verbal fluency for semantic category items correlates with other measures indicating deterioration of semantic memory; (2) the extent to which DAT patients with disturbed comprehension show a co-occurrence of impairments involving loss of cueing, consistency of errors, and preserved superordinate over subordinate knowledge; we will argue that the pattern of results indicates that DAT patients manifest a semantic *storage* disorder; and (3) the assumption that in patients with such semantic storage disorders, off-line semantic cueing and on-line semantic priming will produce the same results.

Patients were initially examined on a detailed battery which looked for impairment of visual perception. Those who were found to be perceptually impaired were excluded from further testing. The same approach was used to exclude patients with impairment of auditory or written work perception, as well as impairment of phonological speech output. This approach, outlined in detail in Chertkow *et al.* (1989), is summarized in Part 1 of the Methods and Results section. Any dementia patients who showed no impairment of tests of sensory 'input' or speech 'output' and yet were anomic, might be unable to name objects because of a conceptual disturbance—the objects could not be labelled because they were no longer associated with a fully specified description in semantic memory. We demonstrate a clear item-specificity on different tests of object comprehension in each of the patients, consistent with this interpretation.

In Part 2, the results of tests of verbal fluency on 8 semantic categories are presented and compared with the extent of semantic memory loss previously demonstrated through direct testing in Part 1. In Part 3, the results of verbal probe questions for general and detailed knowledge of concept items, as well as testing for the efficacy of semantic cueing for items our test patients were unable to name, are given. Results of these tests allow us to see whether loss of cueing occurs concurrently with (1) loss of detailed (but not superordinate) knowledge, and (2) consistency of responses in patients with semantic memory impairment. If this proved to be the case, we would provide support for one of the criteria indicating a semantic storage impairment proposed by Shallice and Warrington. In Part 4, we examine the results of on-line semantic priming on a lexical decision task, and compare the results of semantic priming with those of off-line semantic cueing. We will thus be able to determine if these two tasks are indeed equivalent in nature.

METHODS AND RESULTS

Subjects

We screened 42 patients with probable dementia of Alzheimer's type (DAT) Data will be presented only for the 10 subjects that met the criteria of Part 1 below, with accompanying converging evidence of a semantic memory disturbance. The remaining 32 patients were excluded because of clear impairment of visual perception, undue perseveration, inability to consistently understand the task requirements, or inattention.

The selected 10 patients all had probable DAT by NINCDS-ADRDA criteria (McKhann *et al.*, 1984). They were 7 women and 3 men who met the following requirements: English-speaking, Hachinski scores less than 4 (Hachinski *et al.*, 1975), no clinical evidence of focal brain disease on neurological examination, adequate vision and hearing, cooperative and consenting. The patients were screened to exclude any patients with alexia, perceptual agnosia or apraxia on mental status testing.

The patients, all of whom were over the age of 60 yrs, had a mean age of 76.3 yrs and a mean educational level of 11.6 yrs. Minimental state examination (Folstein *et al.*, 1975) scores were below 25, mean 17.3, range 8–24. Scores on the 60-item Boston Naming Test (Kaplan *et al.*, 1983) were 30 or lower (mean 19.8, range 9–29).

Control subjects

A group of 10 normal elderly individuals served as control subjects (7 women and 3 men, aged 67–87, mean 73 yrs). Their educational level was 11.2 yrs on the average. Their scores on the 60-item Boston Naming Test were greater than 30 (range 33–57, mean 46.1), all falling within a normal range for their age (LaBarge *et al.*, 1986). None had a history of neurological illness or mental decline, and all were normal on neurological examination.

Part 1. Perceptual and semantic memory testing

Perceptual testing

Perceptual testing on our 10 patients provided no evidence of impairment of visual perceptual processing, auditory processing, or comprehension of written words that would interfere with their ability to access semantics. This approach, discussed in detail in Chertkow *et al.* (1989), will only be summarized here. Tasks to determine intact visual and auditory perception were as follows.

Reading test A total of 150 single words was presented on 3×5 inch cards to be read aloud Word stimuli consisted of 150 concrete picturable noun exemplars, drawn from 8 semantic categories (15 items each) plus 30 miscellaneous objects. Categories were vehicles, clothing, furniture, tools, fruit, vegetables, animals and body parts. Items in each category were drawn from Battig and Montague (1969) and Rosch

(1975) and were chosen to include both high and low frequency values (Kučera and Francis, 1967) and prototypicality

Lexical decision task. The words used above in the reading test were administered at a second session to test lexical decision ability; 150 pronounceable nonwords were created by altering two letters of each word, maintaining similar bigram frequencies.

Perceptual object decision task. Line drawings of 20 simple household objects and 20 nonobjects designed to incorporate real object parts (drawn from a group designed by Kroll and Potter (1984)), were presented randomly. Subjects had to indicate that a drawing represented a real or an unreal object.

Mixed category word to picture matching test. Subjects were presented with line drawings of the 150 items used in the reading task, taken from the pictorial stimuli of Snodgrass and Vanderwart (1980). These pictures were displayed in groups of 5 items arranged vertically in a column on a card. Each item was drawn from a different one of the 8 semantic categories or the miscellaneous category. An attempt was made to have the items on a card share basic perceptual features. These 31 mixed category (MC) cards were presented consecutively (Butterworth *et al.*, 1984), and subjects were asked to point to the picture named by the examiner. The position of the target in the column was randomly assigned. All stimuli were selected and prescreened on normal subjects such that at least 95% of normals identified each picture correctly.

Western Aphasia Battery (Kertesz and Poole, 1974). This was administered to each patient in the standard manner. Results on the sections measuring Comprehension, and Repetition, and the fluency rating on the Spontaneous Speech section were compared with normal controls.

Results and Discussion

As discussed in the Introduction, our goal was to delineate a subgroup of DAT patients showing no significant impairment of perceptual processes leading to semantic memory access. Our criteria for ruling out visual and auditory perception problems required a DAT patient to demonstrate performance equal to the control group on all 5 of the above tasks (*see* Table 1). Each of the 10 DAT patients considered below met those criteria.

The test results of object decision and mixed-category matching of words to pictures provide evidence that these dementia patients had no difficulty in carrying out visual processing of pictorial stimuli up to a relatively high perceptual level, but imply little about the state of semantic memory itself. Since distractors in the picture-matching test were all from different semantic categories, success on this task would be possible even if only superordinate category knowledge of the items was retained, which is often the case even in dementia patients with severe loss of semantic memory (Warrington, 1975). The results therefore provide evidence of adequate visual and auditory perceptual processing up to the level at which meaning is derived from the input.

Semantic memory testing

It is unclear whether there is a single task which accurately measures semantic memory function. As discussed in the Introduction, one measure which seems to indicate semantic memory loss in a more direct

TABLE 1 PERFORMANCE OF DAT PATIENTS AND CONTROLS ON PERCEPTUAL AND LANGUAGE TASKS (% OF CORRECT ANSWERS)

	Reading words	*Lexical decision*	*Object decision*	*Word to picture match (mixed category)*	*Spontaneous speech (fluency)*
DAT patients (n = 10)	97 8	96 5	97.5	99 0	93 0
Controls (n = 10)	99.2	98.4	98 7	99.3	95.2

manner is the demonstration of an item-to-item association between comprehension loss and anomia (Huff *et al.*, 1986). We planned to test item comprehension by use of a second word-to-picture matching procedure. As Butterworth *et al.* (1984) had pointed out, if distractors were not only perceptually similar, but also originated from the same semantic category, the subject could no longer merely use general residual knowledge of the structure of category items to carry out the matching task. Patients would fail to match correctly if they had lost precise semantic knowledge for the meaning of a verbal label or meaning of a pictured item. We therefore adapted their 'same category' word-to-picture matching task, using the identical target items as in the previous tasks. Failure in this task could then be compared with picture naming for the same items, to assess whether errors in comprehension and production conformed to an item specific deficit.

Methods

Picture naming task. The 150 pictures used in the Reading task and the previous Word to Picture matching task were presented at a separate session 1 wk later to assess picture naming. All items were prescreened such that 95% of normal age-matched controls could name each picture. Four pictures were discarded on this basis.

Same category word to picture matching test. A second set of picture cards was created using the same pictorial stimuli used in the previous naming and matching tasks. Each card in this set contained 5 items. Pictures were presented from within the same semantic category (SC), arranged vertically in a column (Butterworth *et al.*, 1984). Subjects were again asked to point to a labelled target item, the position of the target in the column being randomly assigned. All stimuli were again prescreened and selected such that 95% of normals correctly responded to each item; 5 items were discarded on this basis.

Results

Picture naming test. In the naming task, the controls correctly responded to 144/146 items (98.6% correct). The DAT patients showed a clear anomia on the picture items. They named a mean of 105.1/146 items (71.9%) correctly, range 90–128, and were significantly worse than the control group ($t = 8.54$, $P < 0.001$). There were 15 exemplars for each of 8 specific semantic categories. Considering only these categories, patients named a mean of 10.3/15 items per category, equivalent to 68.7% of pictured objects presented.

Same category word to picture matching. The results (Table 2) suggest that DAT patients were much more impaired on same category (SC) matching than on the previous test of MC matching. While controls performed well on the SC matching test, with a mean of 142.1/145 (98.0%) correct, DAT patients made considerably more errors (117.2/145 (80.8%) on average; $t = 6.05$, $P < 0.001$). Because distractors are from within the same semantic category, this task is in fact a name recognition task demanding use of specific knowledge within semantic memory.

TABLE 2. PICTURE NAMING AND WORD-TO-PICTURE MATCHING WITH SAME CATEGORY (SC) DISTRACTORS

	Picture naming	*SC word-to-picture match*
Controls (10)	144/146 (98.6%)	142.1/145 (98.0%)
DAT patients (10)	105.1/146 (71 9%)	117 2/145 (80 8%)

Analysis by items. The relationship between performance on the naming and the SC matching task (testing name recognition) was examined by comparing each patient's errors on corresponding items in the two tests (Huff *et al.*, 1986). Out of 1171 items for which the correct picture was chosen despite semantic distractors on the SC word-to-picture matching task, the corresponding picture was correctly named, at a separate session, in 84.9% of cases. In contrast, out of 280 items showing incorrect pointing on the word-to-picture matching task, only 21.4% were named correctly at the later session (Table 3). Comparison of the naming scores indicated that the percentage was much higher for items correctly matched on the SC matching task than for those items incorrectly matched. A t test ($t = 9.42$, $P < 0.001$) confirmed that this difference was indeed highly significant, representing an item-specific correspondence.

This item-to-item correspondence between loss of name production and name comprehension represents an association of deficits at the level of individual concepts. Such a finding is consistent with the possibility that the disturbance is at a single functional level, namely loss of information due to a storage disorder is responsible for failure on both tasks. This contrasts with the pattern of results obtained in patients with aphasia, where the inconsistency of the disturbance with respect to individual items has been taken to signify impaired access to an intact semantic memory store (Butterworth *et al.*, 1984).

TABLE 3. NAMING PERCENTAGES FOR DAT PATIENTS AS A CONDITION OF PERFORMANCE ON SAME CATEGORY (SC) WORD-TO-PICTURE MATCHING

	No of items	*No. named*	*Percentage named*
Items correct on SC match	1171	995	84.9%
Items incorrect on SC match	280	60	21.4%

Discussion

The goal of the experiments in Part 1 was to exclude the possibility of significant perceptual deficits in our patient group, to establish in these DAT patients that a semantic memory deficit existed, and to begin to evaluate its nature. We demonstrated that the DAT patients had an item-specific loss of knowledge manifested by an item-to-item correspondence between loss of name comprehension (failure on same-category word-to-picture matching test) and name production (name test). Our findings confirm those of Huff *et al.* (1986) for a similar DAT population. We can conclude from the results of the experiments in Part 1 that our 10 patients with DAT had converging evidence of semantic memory deterioration.

Part 2. Verbal fluency and semantic loss in DAT

In this section verbal fluency on semantic category listing tasks in our patients will be examined in order to assess to what degree this measure is impaired due to deterioration of semantic memory. The same categories whose exemplars were previously tested for picture naming and word comprehension will be studied using the verbal fluency task. In order to compare fluency with some more direct measurement of semantic memory degradation, we also administered verbal probe questions (Warrington, 1975) testing detailed and specific knowledge of each item used in the studies in Part 1. We make the assumption that correct and incorrect voluntary answers to such probe questions reflect the degree of semantic loss. We can then go on to establish whether loss of verbal fluency correlates with this measure.

Methods

Verbal fluency. Patients were tested for verbal fluency in producing exemplars of the 8 semantic categories that were used in Part 1. Patients were asked to list verbally all the items in each category which they could think of; 1 min was given for each category. Testing was carried out at a separate session from Part 1, at least 1 wk later.

Semantic knowledge probes Our method of using probe questions, adapted from Warrington (1975), has been presented elsewhere (Chertkow *et al.*, 1989) and will therefore only be summarized here. Of the pictorial items used in Part 1, 130 were presented to the subject in a separate session 1 wk after initial testing. At a second separate session, the corresponding written word label was administered. Patients were asked not to name the word or picture aloud. They were given forced-choice questions regarding the item, and instructed to answer each probe question. Six questions probing detailed knowledge of the items were administered while the patient viewed each of these presentation materials (words and pictures). Three questions concerned perceptual attributes (e.g. (item = saw) 'Is the tip made of metal or wood?', 'Is it sharp or dull?'). Three more questions were given regarding functional or contextual aspects of the item (e.g. 'Do you cut things with it or lift with it?', 'Is it used on a piece of wood or on stone?'). Questions were again prescreened on normals such that any question not answered correctly by 90% of age-matched controls was discarded and replaced.

Results

Verbal fluency. Control subjects listed a mean of 14.9 items per category (Table 4). There was a wide variation in exemplars generated in different semantic categories. Controls produced a mean of 21.2 body parts, for example, and 16.5 animals, while able to list only a mean of 10.8 tools. These numbers are similar to other published studies (Borod *et al.*, 1980).

TABLE 4 VERBAL FLUENCY—GENERATION OF SEMANTIC CATEGORY EXEMPLARS IN 1 MIN

	Controls (n = 10)	*DAT (n = 10)*
Animals	16.1	5.7***
Vehicles	12.0	5.0**
Fruit	12.0	5.3**
Body parts	21.2	8.8***
Vegetables	13.2	4.2**
Tools	10.8	3.1**
Furniture	15.1	5.6*
Clothing	18.7	6.5**
Mean	14.9 items/category	5.5 items/category

The t test demonstrates significantly less category exemplars for each semantic category in DAT patients vs controls, at the following P values: * $P < 0.005$, ** $P < 0.001$, *** $P < 0.0001$

The DAT patients had a marked impairment, as expected, in their ability to generate exemplars of semantic categories. They produced a mean of 5.5 items per category, range in patients being 2.3–12.5. For every category, this represented a highly significant impairment of category fluency in comparison with the performance of normal controls (*see* Table 4). In terms of specific categories, patients were able to produce a mean of 8.8 body parts, 5.7 animals, and 3.1 tools. More body part names were produced than other category exemplars, perhaps due to visual self-cueing. Fewer tools were produced than other category exemplars, presumably due to the lack of familiarity with tools in the women tested. Otherwise, a close clustering of category fluency results was noted in the 10 patients (i.e. all other category means were between 4.2 and 6.5 exemplars produced in 1 min).

We noted in Part 1 that when asked to name the pictures of 15 items in each of the 8 semantic categories, the DAT patients were able to name a mean of 10.3/15 items in each category. In 9 of the 10 patients, the mean number per category listed was markedly less than the mean number (of 15 items) that were named. Naming was superior to listing in each category for these patients, as it is in normals. DAT patients were able to name 71.1% of the pictures overall, while controls were at ceiling on the naming task. On verbal category fluency, the DAT patients listed overall only 40.0% as many items as the controls. While the two tasks are not directly comparable, it certainly appeared (as would be expected) that DAT patients had greater difficulty with a less constrained task such as verbal fluency, than with a constrained task such as picture naming.

Semantic knowledge probes. DAT patients made significantly more errors in their answers to probe questions of detailed perceptual and functional attributes than did controls. DAT patients were correct on only 87.3% of questions (mean number of errors = 177/1392 questions, SD = 90.8), vs 98.4% correct for controls (mean number of errors = 22.2, SD = 10.8) ($t = 5.47$, $P < 0.001$). The DAT patients appeared to be much more impaired in their answers concerning biological (animals, fruits, vegetables) than nonbiological categories. Riddoch *et al.* (1988) explain this pattern of results as being due to the greater structural and conceptual similarity between the items within a biological category. If we consider only these three categories, the DAT patients were able correctly to answer only 68.2% of detailed questions, compared with 96.4% correct for controls. Given that a simple guessing strategy would produce 50% correct answers, it is clear that this represents a marked impairment in DAT patients. Even within a semantic category, considerable variability was noted. Some items were answered entirely correctly, while others showed marked loss of knowledge as measured by probe questions.

The patients showed significantly greater inability to answer detailed probe questions concerning items whose pictures they were unable to correctly name. This was to be expected, since a semantic deficit is regarded as central to the naming disorder in dementia. On correctly named items, the 10 patients yielded a mean of 0.44 probe errors per item on the 12 questions (range 0.09–0.75). On the unnamed items, patients produced a mean of 3.7 errors per item (range 1.5–5.2) ($t = 15.9$, $P < 0.005$).

The 130 items for each patient were divided into 'intact' and 'degraded' lists, according to the answers on probe questions. If 3 or more errors were made in answering the 12 questions (6 for the word, 6 for the picture), the item was marked as degraded. Errors had to occur in both word and picture presentation conditions. We take this to be a conservative estimate of semantic impairment. Since 6 of the 12 questions could be answered correctly by a simple guessing strategy, a 50% loss of detailed attributes would be required to result in an item being classified as degraded.

Out of 130 items thus probed, DAT patients showed degraded information by these criteria on a mean of 31.1 items (range 14–56), representing 23.7% of the total number. In comparison, on these same 130 items DAT patients were anomic on a mean of 28.6% of the items. Anomia (Table 5) was therefore slightly more extensive than this relatively crude measure of item knowledge loss. Most (88.1%) of the items that were classified as being degraded were also unnamed. Of interest, 10.1% of the items assessed as being 'intact' on probe questions were still not correctly named.

Fluency and semantic degradation. Our primary concern was to analyse to what degree the impairment in verbal fluency directly reflected loss of semantic memory storage as opposed to impaired search procedures. If verbal fluency was limited only by impairment of search procedures, then it would be expected that patients might be able to generate category members on the fluency task irrespective of whether that member was semantically intact or degraded. We demonstrated above that, in our patients, anomia for a particular item was significantly associated with its being semantically degraded. The listed category exemplars were therefore compared with the results on the Picture Naming test. It was found that of a total of 442 items listed (10 patients × 5.5 mean items per category × 8 categories), only 30 (6.8%) represented

TABLE 5 NAMING OF ITEMS IN DAT PATIENTS RELATIVE TO THEIR STATE OF PROBE INTACTNESS (PERCENTAGE OF TOTAL ITEMS IN PARENTHESES)

	No. of items	*No named*	*No. unnamed*
Intact items	980	881 (89.9%)	99 (10.1%)
Degraded items	311	37 (11.9%)	274 (88.1%)

items which had been unnamed by those patients on the Picture Naming test. When those items were probed, it was found that only 6 of the 30 were degraded, while 24 were in the group of items associated with anomia which were nevertheless intact on probe questions. Hence in only 1.3% of cases did a patient generate a category exemplar which was semantically degraded. Semantic loss clearly limits verbal fluency in our patients (Table 6).

Is semantic memory loss the sole component limiting verbal fluency? We have established above that items which were successfully named on the Picture Naming test showed few probe errors, a mean of 0.44/12 per item. This suggests that the bulk of named items are semantically intact, and should therefore be potentially available for listing on the verbal fluency test. In fact, Table 6 shows that this was not the case in 9 of the 10 patients. Patients listed only a mean of 47.8% (range 24–92%) of the items they were able to name on the Picture Naming task. Some additional component of the search strategy might therefore be impaired in these patients.

TABLE 6. CATEGORY LISTING AND PICTURE NAMING IN 10 PATIENTS WITH DAT

Case	*Total listed (A)*	*No. of unnamed items listed (B)*	*No. of these with degraded store (C)*	*Named items listed (A−B) = D*	*Total named of 120 (E)*	*Total D/E (%)*
1. (D.C.)	40	4	0	36	98	36.7
2. (S.B.)	20	0	0	20	82	24.3
3. (A.T.)	90	10	5	80	87	91.9
4. (C.R.)	61	3	0	58	104	55.7
5. (J.M.)	68	0	0	68	100	68.0
6. (E.S.)	35	4	0	31	74	41.9
7. (T.A.)	36	3	1	33	69	47.8
8. (A.C.)	27	3	0	24	86	27.9
9. (H.R.)	46	2	0	44	74	59.4
10. (K.F.)	19	1	0	18	75	24.0
Total	422	30	6	422	849	37.80 (mean)

Discussion

Verbal fluency and object naming both deteriorate in patients with DAT. Patients with a 1 yr history of DAT listed fewer exemplars of animal names than did normal controls (10.61 vs 18.59 in controls), and named 86.3% fewer animals on confrontation naming (Bayles and Kaszniak, 1987). By their third year of illness, DAT patients were producing 5.52 animal exemplars in 1 min, similar to the patients in this study.

Generating category exemplar names in a limited time is a complex task which depends on the integrity of the semantic system as well as effective search strategies. While its impairment in cases of both focal and generalized brain damage has been well documented (Milner, 1964; Benton, 1968; Rosen, 1980; Bayles and Tomoeda, 1983), it is usually unclear exactly which aspects of the task cause limitation in a particular subject's verbal fluency. Some researchers (Huff *et al.*, 1986; Flicker *et al.*, 1987) have made the reasonable assumption that measuring verbal fluency impairment in DAT constitutes a test of semantic memory loss.

We have demonstrated that semantic memory impairment does indeed provide a definite constraint on performance in a category fluency task. Items that were semantically impaired were almost never generated as category exemplars on the verbal fluency task. There are, however, additional limitations on verbal fluency which are distinct and separate from the semantic memory impairment, and might be classed as impairments of 'semantic search'. While semantic degradation in our patients is more marked for biological categories, all categories (excluding tools and body parts) displayed similar verbal fluency. There thus appear to be additional limitations which inhibit category search, unrelated to semantic deterioration itself.

Since two factors (semantic impairment and search impairment) are both present in our mild DAT patients, the validity of using verbal fluency as a direct measure of semantic memory impairment in DAT is questionable.

Part 3. Superordinate knowledge, semantic cueing and consistency of responses

In this section, we will examine the results of verbal semantic cueing in picture naming in our patients, to determine whether loss of cueing is a feature of the anomia. We will then repeat the picture naming task, and look for evidence of consistency of response with the previous naming battery. Finally, we will compare probes of superordinate knowledge and more detailed knowledge of concept items in our patients. From these results we will be able to assess whether loss of semantic cueing occurs concurrently with preservation of superordinate knowledge and consistency of item anomia in these patients, as predicted by Shallice (1987).

Methods

Semantic cueing. In the object naming test in Part 1, patients were successfully able to name an average of 105.1/146 items. In those cases where the patient was unable to produce the object's name, a semantic cue was offered whilst the patient studied the picture. The cue consisted of the imageable word which was most highly associated with the item, drawn from tables of word association norms (Postman and Keppel, 1970). The word was embedded in a sentence (e.g., picture of a 'lion'—cue: 'It is like a tiger'.). Cueing results were recorded. No time limit was given.

Picture naming task. The 146 pictures used in the picture naming task of Part 1 were administered again 1 month later. Pictures were presented one at a time in a random order with semantic categories mixed together, and patients were asked to name the item. Results were compared with the previous naming task to examine consistency of correct and incorrect responses.

Superordinate knowledge probes. At the same time the detailed probe questions were administered (Part 2), a preliminary question concerning superordinate category membership was also administered (e.g., 'Is this a tool or clothing?'). This was carried out for each of the 130 items in specified semantic categories.

Neuropsychological evidence suggests that the mental representations of semantic concepts are organized hierarchically with different levels of specificity. Object concepts may be classified at a very general level termed the *superordinate*. Category labels such as 'animal' and 'furniture' are examples of such a higher level categorization (Rosch, 1975). Objects are more usually defined at a more concrete level termed the *basic level* where they share the most attributes, overlapping structural features, and gestures. 'Dogs' and 'chairs' are examples of basic level descriptions (Rosch *et al.*, 1976; Jolicoeur *et al.*, 1984). Object concepts may be most specifically defined at a *subordinate* level of categorization which specifies the subtype of the concept being encountered. 'Dalmatians' and 'armchairs' would be subordinate level labels.

Other researchers have adopted a similar terminology with respect to the semantic impairments in brain-damaged patients. Generally, superordinate knowledge is taken to include understanding of the general semantic category of concepts, while subordinate knowledge would refer to knowledge of the specific attributes and associations of object concepts (Warrington, 1975; Shallice, 1987).

Results

Semantic cueing. Eight of the 10 patients were tested for semantic cueing. The 8 patients were anomic on a mean of 41.0 (range 18–56) of the 146 items in the battery. When semantic cues were administered for these items (total 328), there was a remarkable lack of effect. Only 43 previously unnamed pictures could be named on presentation of a semantic cue, while 285 items remained unnamed. Semantic cueing was thus found to be ineffective in 86.9% of trials.

Most, but not all of the anomia of DAT patients, we have argued, reflects semantic loss. The unnamed items for which cueing *was* effective (13.1% of the total) were therefore examined in relation to their semantic intactness according to verbal probes presented in Part 2. Any items showing 3 or more (out of 12) errors on probe questions had previously been designated as 'degraded' while others were termed 'intact'. Of these 43 items in total which benefited from cueing, 25 were found to be semantically intact, and only 18 were degraded. Therefore, if the 25 intact items are excluded, it becomes evident that semantic cueing was only effective in 5.4% of cases where the item was not only unnamed, but was semantically degraded.

Picture naming task. On the initial battery, the 10 patients with DAT were anomic on a total of 409 responses (mean 40.9), out of a battery of 146 items. When the test was repeated, the same items were unnamed in 378 of cases, 92.5% of the initially unnamed items being again unnamed on the second trial. The coefficient of consistency, kappa, was calculated for each of the 10 patients. Kappa measures may vary between −1 (complete inconsistency) and +1 (complete consistency), with kappa greater than 0.65 generally being taken to indicate a high degree of consistency (Fleiss, 1981). For each of our patients, kappa was found to lie in the range +0.75 to +0.91, confirming the high consistency of the performance

Superordinate semantic knowledge probes. When answers for the word and picture conditions were combined, questions regarding the superordinate category were answered equally well by DAT and controls (Table 7). Most errors were in differentiating fruits from vegetables. Excluding fruit/vegetable confusions, the DAT patients were correct 98.2% of the time. Even when considering only the items for which patients were anomic, there was still marked preservation of superordinate knowledge. For anomic items, the patients were able to identify correctly the superordinate category in 96.2% of cases.

TABLE 7 MEAN PROBE QUESTIONS ANSWERED ERRONEOUSLY BY DAT PATIENTS AND CONTROLS

	Superordinate questions (total 252)	*Subordinate questions (total 1392)*
DAT patients	8.8 = 3.5%	177.4 = 12.7%
(10)	(SD 3.1)	(SD 90.8)
Controls	3.2 = 1.2%	22.2 = 1.6%
(10)	(SD 1.5)	(SD 10.8)

Discussion

In Part 2 we noted that the 10 patients with DAT all displayed a marked loss of *detailed* probe knowledge. We now present evidence that in contrast to this they performed at normal control level of accuracy in answering *superordinate* probe questions about item pictures and words. Knowledge of category membership therefore remained relatively preserved and at ceiling both for patients and controls. This outcome suggests that while dementia patients can demonstrate knowledge of category membership (Diesfeldt, 1985), such knowledge does not preclude a significant deterioration of semantic memory. In Part 3 we have demonstrated that these same patients have remarkable consistency in their anomic responses, when repeat measurements are taken a month later. In addition, semantic cueing for these unnamed items was effective in only 5.4% of instances, if the item was not only unnamed but semantically degraded. Relative loss of more detailed knowledge (with preservation of superordinate knowledge), consistency of response and loss of semantic cueing therefore do occur together in these patients with direct evidence of semantic storage loss, consistent with the position advocated by Shallice (1987).

In Part 2 we noted that 10.1% of the semantically intact items remained unnamed by our patients. In Part 3 it is noted that in 25 out of 328 cases (7.6%) where an item was unnamed, there was effective semantic cueing with an associated word, and the item proved to be intact on semantic probes. While this small subgroup may be due to chance variation in both cases, it raises the possibility that although most of the anomia in these patients occurred on the basis of semantic memory deterioration, roughly 5−10% of the anomia may have been due to an additional impairment in the ability to access the phonology of the word label for a particular picture. In such cases, a semantic cue might be very effective by aiding contextual self-cueing, similar to that commonly seen in aphasic patients (Benson, 1979). The semantic probes would indicate intact semantic knowledge for those items.

Part 4. Semantic priming and cueing

We wished to examine the hypothesis that tests of semantic priming in our patients would provide results similar to tests of semantic cueing. We therefore designed an on-line experiment of semantic priming on a lexical decision task, using the same stimuli as had been used in Part 3 for the semantic cueing task. Only 6 of the DAT patients were able to cooperate adequately to carry out a speeded lexical decision task, and data are therefore presented only for these 6. The method will only be briefly outlined here (for details, *see* Chertkow *et al.*, 1989).

Methods

A test of lexical decision, using semantic priming, was administered to 6 of the DAT patients and 10 controls participating in Parts 2 and 3. Fifty pairs of associated words were used as priming stimuli. Targets consisted of words drawn from the 8 semantic categories used in Parts 1–3. For each target, the associated prime word consisted of the most highly associated concrete imageable word (Postman and Keppel, 1970). The stimuli were constructed such that the associated priming word was identical to the word used for cueing in Part 3 (e.g., 'tiger' and 'lion'). For each associated pair, two unassociated word pairs were also created using the same target words as before, but replacing the priming word with an unrelated item from a different semantic category, balanced with the prime for word length and frequency. All words used were one or two syllables and less than 10 letters. Four other pairs were created with nonword members in prime, target or both positions.

At the beginning of each trial, the word 'READY' appeared, centred on the screen for 550 ms. This was then removed and after a 500 ms delay, the prime stimulus appeared. The subject's response into a microphone (yes or no) erased the prime, and after an interval of 500 ms, the target stimulus appeared, and again the subject responded. After a 4 s intertrial interval, the sequence was repeated. Reaction times for the lexical decision task were calculated, along with errors. The priming effect for each item was obtained by subtracting the response time for a particular target when preceded by an associated word from the response time when preceded by a nonassociated word.

Results

Latency analysis. Controls (n = 10) showed a mean reaction time of 831 ms (SD = 228 ms, range 570–1183 ms) for performing lexical decisions on the unprimed targets preceded by unassociated primes. When preceded by an associated prime, they showed a mean of 24.8 ms priming effect. All 10 showed such priming effects, similar to those obtained in previous series with elderly subjects (Howard *et al.*, 1981; Burke *et al.*, 1987). Results are comparable with the many published studies of lexical decision priming in younger populations (i.e., Fischler, 1977*a,b*; Neely, 1977; Seidenberg *et al.*, 1984) which show very similar priming effects. Although there was a trend for greater priming effects to occur in the normal subjects with slower responses, the difference did not approach significance.

The 6 DAT patients all completed the priming experiment. They also displayed a wide range of reaction time (RT) on the unprimed targets, but were slower than normals Mean unprimed RT was 1255.1 ms, ranging from 1070 to 1428 ms. All DAT patients showed priming effects. Total priming effect yielded a mean of 140.6 ms, ranging from 84.7 to 267.6 ms in the patients.

Comparing semantic priming and cueing. In designing the cueing task, care had been taken that the cue words used would be identical to the words used as primes in the on-line semantic priming task. It was therefore possible to extract word pairs from the results of these investigations with the following properties: (1) the item had been unnamed on the naming task, and probes of word and picture in Part 2 showed it to have a degraded representation in semantic memory; (2) semantic cueing had been carried out; (3) the same word pair has been used as a Prime and Target on the semantic priming task and a Cue and Target on the picture naming task. We found (Table 8) 73 word pairs that satisfied these criteria. Inspection of the data suggested that semantic priming and semantic cueing did not produce comparable results when using the same words.

TABLE 8 SEMANTIC CUEING AND SEMANTIC PRIMING IN 6 DAT PATIENTS

	Case						
	6	*7*	*5*	*4*	*3*	*10*	*Total*
Cueing +, priming +	0	2	0	2	3	0	7
Cueing +, priming −	2	0	3	0	0	0	5
Cueing −, priming +	2	5	2	5	12	11	37
Cueing −, priming −	4	3	0	7	5	5	24
No. of pairs	8	10	5	14	20	16	73

If we consider cueing to be positive when it leads to successful picture naming, and priming to be positive when it leads to a shorter response time for performing a lexical decision, then the results are as follows. In only 7 pairs were both positive for cueing and priming, while in another 24 pairs, both were negative. In comparison, the two tests produced divergent results in 42 cases. Of the 61 pairs for which cueing was not effective, priming was negative in 24 cases, but positive in 37 cases. When the correlation was assessed statistically by Kendall's Tau (Fleiss, 1981, p. 118), it was found that the correlation between the two was not significantly greater than zero ($z = 1.8$, $P = 0.0719$). It appears that results from a cueing test do not predict results on semantic priming for the same word pair.

Is it possible that this lack of correlation is merely due to the different sensitivity of criteria for a 'positive' result of cueing and priming? After all, a positive priming effect is indicated by any degree of speeded response in the associated condition, while the result of cueing is 'all or none'. If this were indeed the crucial factor, it might be anticipated that prime-target pairs showing the greatest magnitude of priming might also show a positive cueing response, while those showing positive but *low* magnitude priming, would not be associated with effective semantic cueing.

Reexamination of the data does not support this hypothesis. The 10 pairs (out of 44) that showed the greatest magnitude of priming (mean = 1101 ms) were examined. Semantic cueing was effective in only 2 of these pairs. Alternatively, in the 10 pairs showing positive but lowest magnitude priming (mean 60 ms), there were still 2 pairs which demonstrated effective semantic cueing. In no way, then, does the presence, absence, or magnitude of semantic priming appear to predict positive semantic cueing.

Discussion

Semantic cueing and semantic priming on lexical decision share certain features. In both, the subject's response to a target is aided via presentation of a prior stimulus which is semantically related. It might therefore seem reasonable to expect, as Shallice implied (1987), that the two tasks would be similarly affected in brain damage. One would expect that deterioration of semantic memory such as occurs in DAT would affect the two tasks in a similar manner. Contrary to this expectation, we demonstrate that on-line semantic priming on a lexical decision task, and semantic cueing on an object naming task, produce strikingly different patterns of results.

One potential weakness of our method was that the tasks, naming and lexical decision, were different for cueing and priming. Lexical decision was used for the on-line task because the DAT patients had difficulty in limiting verbal output to simple naming responses, causing a high rate of unacceptable responses. In patients who have had both naming and lexical decision tested, however, the pattern of results has been similar for the two tasks (H. Chertkow, D. Bub and A. Bruemmer, unpublished data, 1989). Other possible reasons for the differences will be examined below. What is clear is that results of semantic cueing and semantic priming are not predictive of each other, nor do they constitute interchangeable methods.

GENERAL DISCUSSION

In this paper we have undertaken to examine three issues which arise from a consideration of the tests used to define the presence of a semantic memory disturbance. We began in Part 1 by presenting data from 10 patients with DAT whom we argued showed strong converging evidence of impaired semantic memory for certain items. Tests of visual and verbal perceptual abilities were not significantly different from age-matched controls. Patients were anomic for a wide range of items. Loss of ability to match words to pictures in the presence of semantic distractors, despite excellent ability to match in the presence of unrelated distractors, represented evidence for a comprehension deficit at the semantic level. We argued that the demonstration of an item-to-item correspondence between loss of comprehension of a concept item, and loss of ability to name a picture of that item, constituted further evidence for a critical semantic level disorder. This position is certainly consistent with recent views on the functional impairment of patients with DAT (Bayles and Kaszniak, 1987).

In Part 2, we presented results of tests of 'verbal fluency', the ability to generate semantic category exemplars in one minute. We concluded that there were two probable sources for the marked impairment of verbal fluency in our patients. One source was the deterioration within semantic memory itself, for degraded items were almost never generated in the listing task. The other impairment, we suggested, lay at the level of 'semantic search', a complex and ill-defined procedure which might be affected by attentional problems, motivation, and the ability to devise strategies for breaking down categories into smaller sections for efficient search. Verbal fluency is a useful task for clinical neuropsychological assessment, and has practical utility in questions of diagnosis and lesion localization (Milner, 1964; Benton, 1968; Miceli *et al.*, 1981). It remains, however, a psychologically complex task. Our results suggest that it might be misleading to extrapolate from impairment on such a task to impairment of a specific cognitive processing stage such as that of semantic memory.

In Parts 3 and 4, we examined two hypotheses, one explicit, the other implicit, which arise out of a series of papers by Shallice and Warrington. We found that our 10 patients, all of whom were shown to have a semantic impairment, displayed evidence for the concurrence of all three of the diagnostic criteria specified by Shallice (1987) for a semantic storage disorder. They all showed marked consistency in their errors in naming, a consistency obtained with a month between testing sessions. They all showed loss of detailed semantic knowledge on probe questions, but preservation of superordinate knowledge for the same items. In addition, they all displayed a marked loss of semantic cueing. Associative cues were able to facilitate the naming of otherwise unnamed items in only 5.4% of cases where an item was semantically degraded.

In Part 4, we examined and compared the results of semantic cueing on a test of picture naming, and semantic priming on a lexical decision task. We demonstrated a striking difference in the outcome of the two procedures using the same stimuli on the same patients. There are many clear differences between the two tasks, which might account for these results. Priming uses on-line measures of reaction time, while cueing measures only success or failure in carrying out the naming task. Priming may proceed automatically by spread of associations at the level of the orthographic lexicon (Posner and Snyder, 1975) and even prove effective when the subject is not aware of having seen the prime word (Marcel, 1980). A cueing task operates only through the subject's conscious attempts to search for the answer, and depends on his motivation and intellectual abilities. Priming is produced using only the presentation of word labels, while cueing requires use of a word to aid identification of a picture. We might not be surprised, then, if the results of priming and semantic cueing failed to correspond.

The data presented in Part 4 confirm this lack of correspondence in our patients. While semantic cueing is absent in the majority of items, semantic priming continues to be effective. Not only are words able to prime each other, but the priming appears to occur to a quantitatively greater degree in the DAT patients than in normal controls. Nebes *et al.* (1984) and Chertkow *et al.* (1989) have previously noted that semantic priming in DAT may be preserved and can even occur to a greater degree than in age-matched normals. The reason for this remains unclear. Semantic cueing and semantic priming are therefore not predictive of each other, nor do they constitute interchangeable tasks.

Our data suggest that a clinical symptom such as anomia can originate from a number of different functional disturbances in the same patient. For most of the items tested, the anomia appeared to originate at the level of semantic memory. Deterioration of detailed conceptual knowledge of an item appears to disrupt the patient's ability to name that item's picture. In such cases, semantic cueing will not aid in naming the picture. The item is almost never offered as a category exemplar on a verbal fluency test. Automatic priming experiments may, however, continue to demonstrate the ability of related concepts to act as primes for that item, at least on a task of lexical decision.

There appeared to be a small number of items which were unnamed as well, but for different reasons. For these items (approximately 5–10% of the unnamed items), probes indicated that semantic memory for the concept was relatively intact. Semantic cueing for this group of items did result in correct naming in a majority of cases. These items were also sometimes offered as category exemplars on a verbal fluency task. While chance may account for the appearance of this small subgroup of items, it is equally possible that it represents a second functional impairment, namely the inability of a patient to access the phonological label for that particular word from an intact semantic representation. This impairment is similar to the 'word selection anomia' documented by Benson (1979) in some aphasic patients with left hemisphere lesions.

In conclusion, it is suggested that a close and detailed evaluation of the clinical deficits of patients with dementia of Alzheimer's type can serve to elucidate these methodological issues regarding the evaluation of semantic memory impairment, and lead to a more precise and accurate measurement of their cognitive deficit.

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A MITOCHONDRIAL ENCEPHALOMYOPATHY WITH SPECIFIC DEFICIENCIES OF TWO RESPIRATORY CHAIN POLYPEPTIDES AND A CIRCULATING AUTOANTIBODY TO A MITOCHONDRIAL MATRIX PROTEIN

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SUMMARY

A 15-yr-old boy with mitochondrial encephalomyopathy and NADH CoQ reductase (Complex I) deficiency is presented. Immunoblotting demonstrated specific deficiencies of the 24 kDa FeS protein of Complex I and subunit II of Complex IV. The patient's serum contained an antibody to a specific mitochondrial matrix polypeptide of apparent Mr 41 kDa. The specific polypeptide deficiencies involve products of nuclear (24 kDa FeS protein) and mitochondrial (subunit II) genes and suggest some intergenomic regulation. The relevance of the circulating antibody to the pathogenesis of the patient's Complex I deficiency is discussed.

INTRODUCTION

The mitochondrial encephalomyopathies constitute a complex group of multisystem diseases thought to be due to specific defects in the mitochondrial respiratory chain (Morgan-Hughes *et al.*, 1982, 1987; DiMauro *et al.*, 1985; Petty *et al.*, 1986). About 20% of patients with mitochondrial myopathy have similarly affected relatives and the ratio of maternal to paternal transmission is approximately 9:1 (Harding *et al.*, 1988). The aetiology of the disorder in patients without a family history is unclear but may be acquired or involve spontaneous mutations of nuclear or mitochondrial genes. A viral aetiology has been suggested for the Kearns-Sayre syndrome (KSS), a clinical subtype of mitochondrial myopathy (Berenberg *et al.*, 1977). A number of patients with mitochondrial myopathy have responded well to the introduction of corticosteroids and have deteriorated on any attempt at withdrawal (Shapira *et al.*, 1975; Skoglund, 1979). This suggests that disordered immunity may play a role in some patients. This paper presents a 15-yr-old boy with a mitochondrial encephalomyopathy and a Complex I defect who had a circulating antibody which bound specifically to a single mitochondrial matrix protein. The role of this antibody in the pathogenesis of the disease is discussed.

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This case has been included in recent reviews of patients with Complex I deficiency (Morgan-Hughes *et al.*, 1987, 1988).

CASE HISTORY

The patient (E.C.) was born at term, the first child of nonconsanguinous parents. Between the ages of 10 months and 3 yrs he had 5 episodes of hypoglycaemia and loss of consciousness. There was some failure to thrive and at the age of 11 yrs he had a series of generalized seizures. Neurological examination and a CT brain scan were normal at this stage. Over the next 2 yrs he suffered repeated seizures followed by episodes of right-sided weakness. Repeat CT scan showed a low density area over the left rolandic fissure. His mother who was aged 38 yrs, had had 2 epileptic seizures when aged 25 yrs and had been treated with diazepam, phenobarbitone and sodium valproate. She was taking these anticonvulsants at the time the patient was conceived. There was no family history of any autoimmune disorder.

On examination at the age of 15 yrs the patient's mentation was slow and he had a mild slurring dysarthria with some perseveration. He was of small stature, thin and generally wasted. He had bilateral optic atrophy but no retinal pigmentation, ptosis or ophthalmoplegia. He had bilateral sensorineural deafness. Muscle bulk and power in the limbs were generally reduced and easily fatigued. Tendon reflexes were brisk and plantar responses were extensor. There was marked limb and gait ataxia.

Full blood count, serum electrolytes, liver function tests and chest x-ray were normal. The ECG showed a Wolff-Parkinson-White type-B dysrhythmia. The EEG showed frequent bursts of generalized epileptiform activity, probably arising from a right temporal focus. A high resolution CT brain scan (fig. 1) showed cerebral and cerebellar atrophy with calcification of the basal ganglia. The patient was unable to undertake an exercise test but his resting lactate was elevated at 1.58 mM (normal 0.37 ± 0.1 mM).

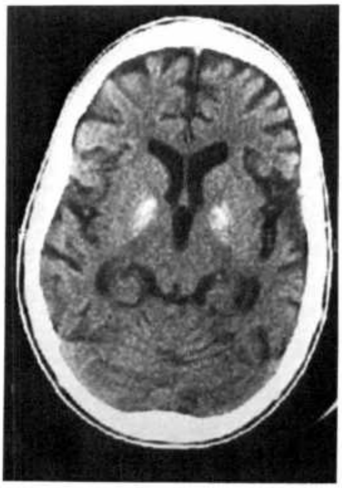

FIG. 1. High resolution CT brain scan of E.C.

A muscle biopsy from the left vastus lateralis (fig. 2) showed 30% ragged red fibres and 4% cytochrome oxidase-negative fibres. There was no excess of neutral lipid. Electron microscopy showed increased numbers of morphologically abnormal mitochondria containing electron-dense matrix material but no paracrystalline inclusions.

Examination of the patient's mother did not show any abnormality and her exercise test was normal. A biopsy taken from her right peroneus brevis, however, showed ragged red fibres.

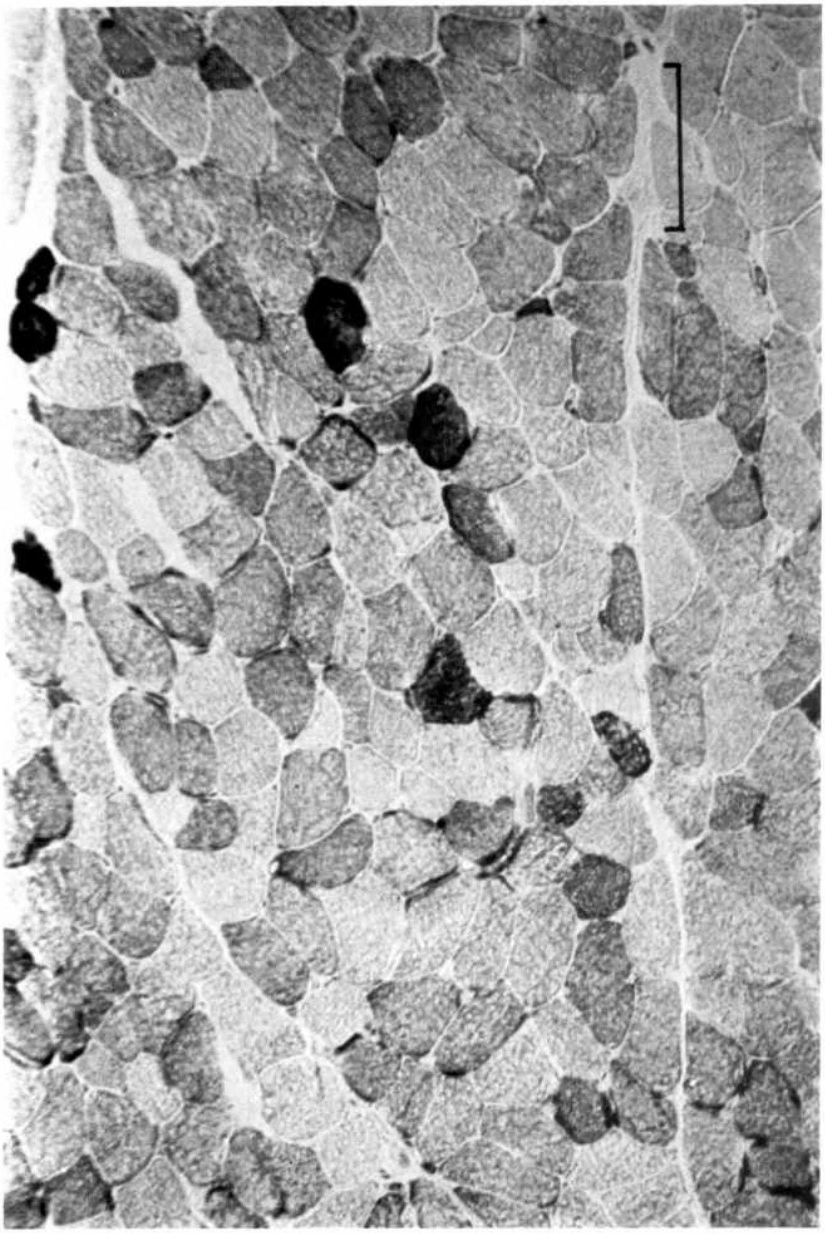

FIG. 2. Gomori trichrome stain of E.C.'s muscle biopsy showing high proportion of ragged red fibres. Bar = 1 μm.

METHODS

Biochemical studies

Isolation of mitochondria from a biopsy of the left vastus lateralis, polarographic studies and cytochrome measurements at 77 Kelvin (K) were performed as previously described (Morgan-Hughes *et al.*, 1977, 1982). Mitochondrial samples were stored at 77 K in the presence of 5 mM aminobenzamidine. Control mitochondria were isolated from the vastus lateralis muscle of 4 patients with no clinical, morphological or biochemical evidence of mitochondrial disease. Postmortem human heart mitochondria were prepared by the trypsin digestion method (Davies *et al.*, 1981), kidney and liver mitochondria by the method of Hayes *et al.* (1985), and brain mitochondria by the flotation method of Booth and Clark (1978).

Gel electrophoresis and immunoblotting

For analysis of Complex I polypeptides, samples were solubilized in 2% (w/v) sodium dodecyl sulphate (SDS), 5% (v/v) mercaptoethanol, 10% (v/v) glycerol and 62.5 mM Tris HCl (pH 6.8) and boiled for 2 min. Complex III and IV polypeptides were analysed from samples dissolved in 5% (w/v) SDS, 2% (v/v) mercaptoethanol, 8 mM urea and 0.25 M Tris HCl (pH 6.8) without boiling. Proteins were separated on 12–16% (Complex I and III) or 12–19% (Complex IV) linear gradient SDS polyacrylamide gels as described previously (Laemmli, 1970; Schapira *et al.*, 1988), prior to electroblotting to nitrocellulose in Tris-glycine-methanol (Towbin *et al.*, 1979). Filters were probed with holoenzyme and subunit specific antibodies to Complexes I, III and IV. Serum containing antimitochondrial antibodies was obtained from a patient with primary biliary cirrhosis (PBC). Sera from E.C., from 2 other patients with Complex I and 24 kDa FeS deficiency, 4 patients with Complex I defects associated with a generalized reduction of Complex I subunits, 2 patients with Complex III deficiency, the patient's mother and 7 controls were also studied for the presence of antimitochondrial antibodies. Human serum was diluted × 100 with phosphate buffered saline (PBS)-0.1% (v/v) Tween-20 for immunoblotting. Bound antibodies were detected with the biotin-streptavidin-horseradish peroxidase system (Amersham). Individual polypeptides were identified in total protein separations by colloidal gold staining and immunoblotting as previously described (Schapira and Keir, 1988).

Two-dimensional electrophoresis was performed by nonequilibrium pH gradient electrophoresis (NEPHGE) (O'Farrell *et al.*, 1977). Samples were dissolved in SDS, Triton X-100, mercaptoethanol and 8 M urea and separated on polyacrylamide-urea tube gels. Tube gels were equilibrated in 5% mercaptoethanol, 2.3% SDS and 62.5 mM Tris pH 6.8 for 30 min before being sealed to the top of slab gels and run in the second dimension through SDS-polyacrylamide.

Membrane fraction preparation

Rat liver mitochondria were isolated by the method of Hayes *et al.* (1985). Separate mitochondrial inner and outer membrane and matrix fractions were isolated using the sucrose density fractionation of Parsons *et al.* (1966). Membrane contamination of the matrix fraction was reduced by centrifugation at 250,000 *g* for 6 h. Malate dehydrogenase (Ochoa, 1955), succinate cytochrome c reductase (King, 1967), and monoamine oxidase (Ragan *et al.*, 1987) were used as markers for matrix, inner and outer membranes, respectively.

RESULTS

Respiratory studies

Polarographic studies and cytochrome measurements are summarized in Table 1. Oxygen uptake rates with NAD-linked substrates pyruvate and glutamate were reduced to 23% and 20% of control mean values, respectively. Succinate and ascorbate + TMPD

TABLE I. STATE 3 RESPIRATORY ACTIVITIES (nmol O. min^{-1}, mg mitochondrial protein^{-1}) AND CYTOCHROME CONTENT (nmol. mg mitochondrial protein^{-1}) OF ISOLATED SKELETAL MUSCLE MITOCHONDRIA

	Pyruvate	*Glutamate*	*Succinate*	*Ascorbate + TMPD*
Control	106 ± 21	98 ± 19	147 ± 63	307 ± 135
E.C.	24	20	153	216
Cytochrome	b	c_1	c	aa_3
Control	0.47 ± 0.05	0.54 ± 0.07	0.72 ± 0.09	0.42 ± 0.06
E.C.	0.266	0.385	0.629	0.161

Control values (n = 4) are mean ± 1 SD.

oxygen uptake rates were within normal ranges. These results indicate a defect in Complex I of the respiratory chain (fig. 3). Levels of cytochromes b, c_1 and c, as determined by low temperature spectra, were mildly decreased, but cytochrome aa_3 was reduced to 38% of the control mean. Muscle homogenate citrate synthase activity was 17.6 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g wet weight}^{-1}$ (control 9.8 ± 2.2).

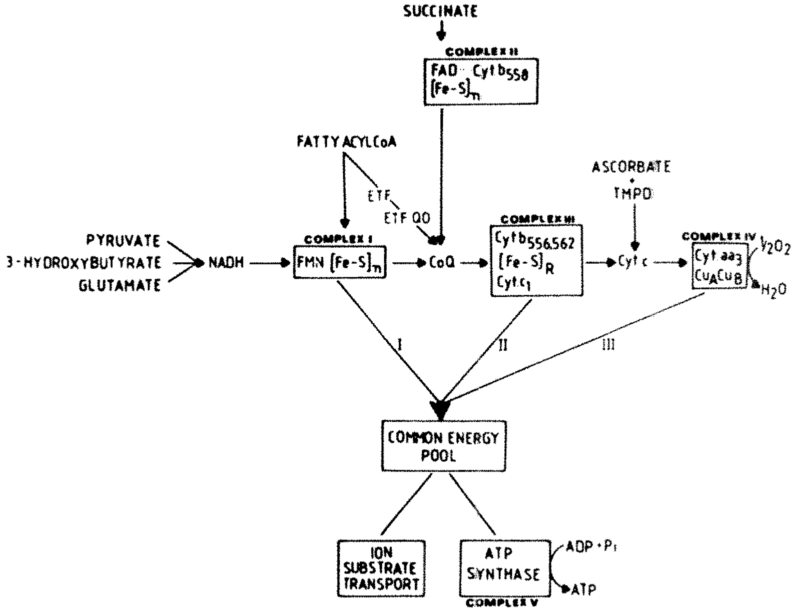

FIG. 3. Schematic representation of the mitochondrial respiratory chain. Reduced NAD-linked substrate but normal succinate utilization rates isolate the defect of Complex I. NADH = nicotinamide adenine dinucleotide (reduced); FMN = flavin mononucleotide; FeS = iron-sulphur; ETF = electron transfer flavoprotein; ETF-QO = ETF-CoQ reductase; FAD = flavin adenine dinucleotide; CoQ = co-enzyme Q; FeS_R = Rieske FeS; TMPD = tetramethylphenylene diamine; Cu = copper; ADP = adenosine diphosphate; ATP = adenosine triphosphate.

Respiratory chain polypeptide studies

Fig. 4 shows immunoblots of the patient's mitochondrial protein with antibodies to Complexes I, III and IV. There is a moderate generalized reduction of all cross-reacting

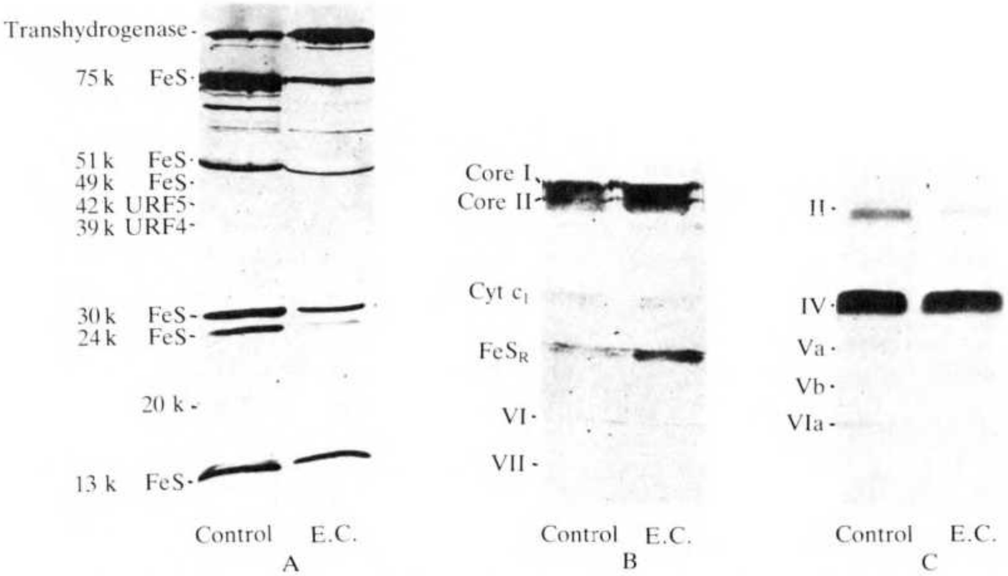

FIG. 4. Immunoblots of 100 μg of control and E.C. skeletal muscle mitochondrial protein; 12 – 16% linear gradient SDS-polyacrylamide gels for Complexes I (A) and III (B) and 12 – 19% gel for Complex IV (C) polypeptides. Holoenzyme antibodies to Complexes I, III and IV were used on the respective blots together with the following subunit specific antisera: 30 kDa, 24 kDa and 13 kDa FeS proteins (Complex I); Rieske FeS protein (Complex III) and subunit II (Complex IV).

Complex I polypeptides with a severe and disproportionate loss of the 24 kDa FeS protein. The human equivalent of the bovine 30 kDa FeS protein has an apparent Mr of 28.8 kDa on Laemmli gels. However, by convention it is still referred to as the 30 kDa protein. Complex I holoenzyme antibody also cross-reacts with a 110 kDa protein thought to be pyridine dinucleotide transhydrogenase, an inner mitochondrial membrane protein which commonly co-purifies with Complex I. The level of transhydrogenase was increased in E.C. Compared with controls, cross-reacting Complex III polypeptides were normal. In Complex IV, bands corresponding to the nuclear encoded subunits IV, Va, Vb and VIa were similar to controls but the mitochondrial gene product, subunit II, was reduced in the patient. The antibodies used did not cross-react with subunits I and III of Complex IV.

Primary biliary cirrhosis (PBC) antibody studies

Immunoblotting E.C.'s muscle homogenate proteins with PBC serum (fig. 5) showed an increase in cross-reacting material per gram of muscle protein when compared with controls. PBC serum is known to contain antibodies directed at mitochondrial antigens outside the respiratory chain, and which include components of the pyruvate dehydrogenase complex (Yeaman *et al.*, 1988). This finding indicates an increase in total mitochondrial mass in E.C. and is consistent with increased citrate synthase activity in muscle

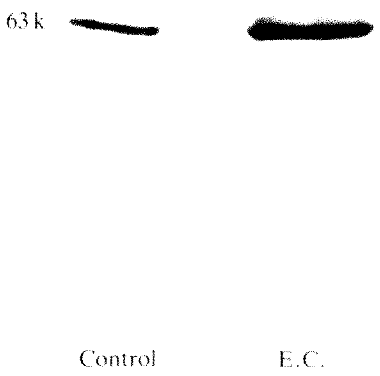

FIG. 5. Immunoblot of 50 μg of control and E.C. skeletal muscle homogenate with PBC serum.

homogenate. An immunoblot of E.C.'s muscle homogenate protein with Complex I antibodies (fig. 6) demonstrated that despite a marked increase in total mitochondrial mass, there was still a severe and generalized reduction of identified Complex I subunits per gram of muscle. The 24 kDa FeS protein was not seen in homogenate blots.

Autoantibody studies

Fig. 7 shows the immunoblot of a series of skeletal muscle mitochondrial preparations with the patient's serum. This series contained 2 controls (C1, C2), E.C. and 2 other

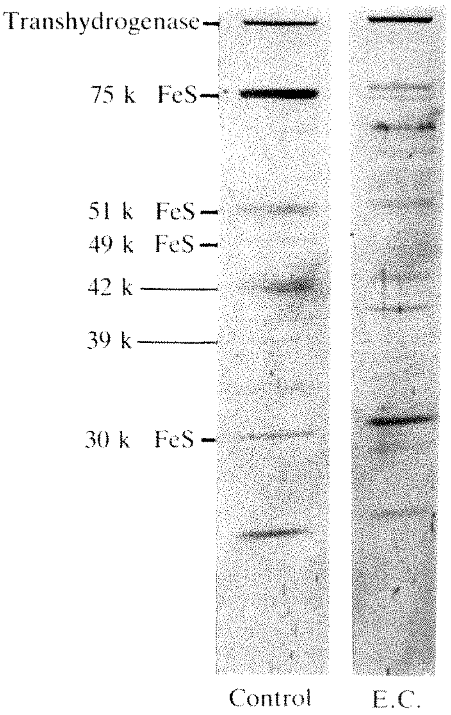

FIG. 6. Immunoblot of 150 μg of control and E.C. skeletal muscle homogenate with Complex I holoenzyme antibody.

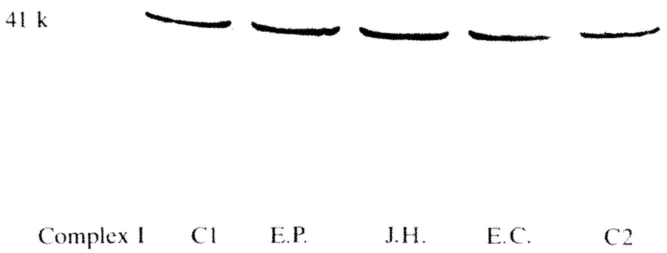

FIG. 7. Immunoblot with E.C. serum against 100 μg of skeletal muscle mitochondria from 2 controls (C1, C2), E.P., a patient with Complex III deficiency, J.H., a patient with Complex I deficiency, and E.C. The molecular mass of the cross-reacting bands was determined as described in the text.

patients with mitochondrial myopathy. These were E.P., a patient with Complex III deficiency and specific reduction of the Rieske FeS protein (Schapira *et al.*, 1987) and J.H., a patient with Complex I deficiency and specific reduction of the 24 kDa FeS protein (Schapira *et al.*, 1988). A single cross-reacting band was present in all tracks. Its apparent molecular mass of 41 kDa was established by colloidal gold staining and

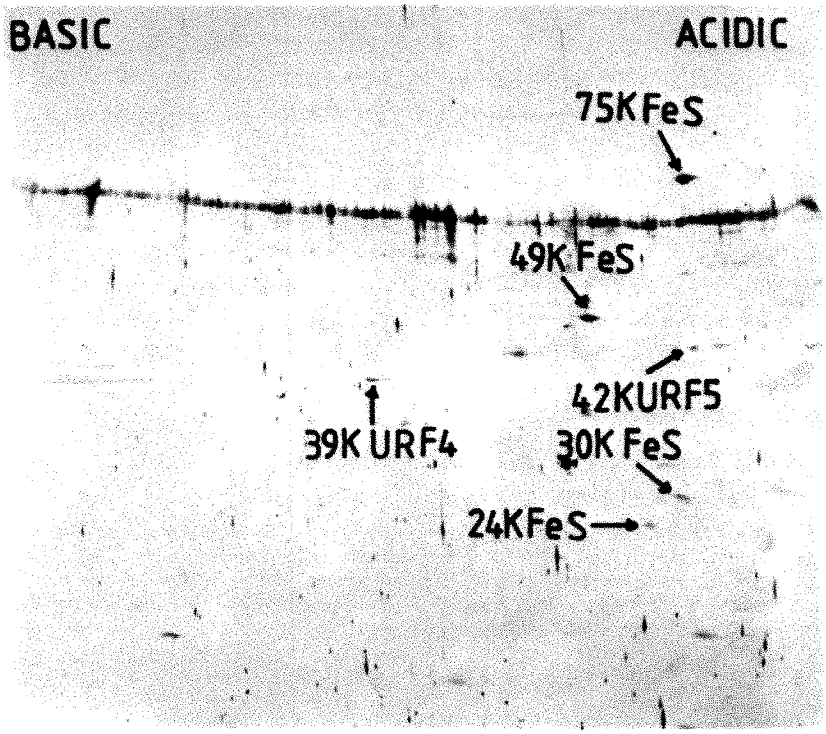

FIG. 8. Two-dimensional NepHge separation of 150 μg of human heart mitochondria transferred to nitrocellulose and immunoblotted with antibodies to the holoenzyme of Complex I and the 49 kDa and 30 kDa FeS proteins.

immunoblotting (Schapira and Keir, 1988). Further immunoblots with the patient's serum demonstrated the 41 kDa antigen in heart, skeletal muscle, liver, kidney and brain mitochondria from human, bovine, rabbit and rat tissues (data not shown). Sera from 6 other cases with Complex I defects, 2 cases of Complex III defects, the patient's mother and 7 controls did not cross-react with any mitochondrial protein.

Identification of the mitochondrial antigen recognized by E.C.'s serum

Two-dimensional separations of human heart mitochondria were transferred to nitrocellulose and probed with antibodies to Complex I (fig. 8). The filter was then reprobed with the patient's serum (fig. 9) to show that the band at 41 kDa seen on 1-D separations represents a single polypeptide and is distinct from those of Complex I. Similar experiments (not shown) demonstrated that the antigen is also distinct from the mitochondrial antigens recognized by PBC serum.

Fig. 10 shows the results of immunoblotting rat mitochondrial outer and inner membrane and matrix fractions with E.C.'s serum. The purity of each mitochondrial subfraction was assessed by marker enzyme analysis (Table 2). The 41 kDa antigen was present in whole mitochondria from rat muscle and liver and also in purified liver matrix fraction (tracks 1, 2 and 3) but absent from the inner and outer membrane

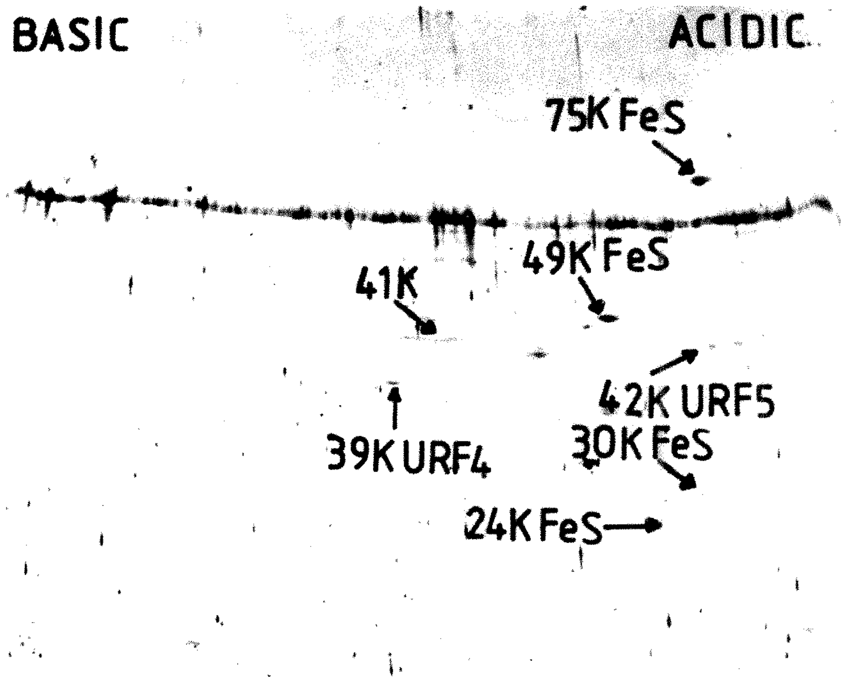

FIG. 9. The immunoblot shown in fig. 8 after further immunoblotting with E.C. serum. A single polypeptide at 41 kDa cross-reacted with the patient's serum.

FIG. 10. Immunoblot with E.C. serum of 50 μg of mitochondria from rat skeletal muscle (1), rat liver (2), and rat liver mitochondrial subfractions of matrix (3), inner membrane (4) and outer membrane (5). Other tracks contained purified beef heart Complexes I and III (track 6), Complex IV (track 7) and Complex V (track 8).

TABLE 2. MITOCHONDRIAL SUBFRACTION ENZYME ACTIVITIES

	MDH	*Succ.cyt.c.red.*	*MAO*
Liver mitochondria	7151	19.0	5.2
Matrix fraction	8642	0.7	0.7
Inner membrane	213	10.0	1.6
Outer membrane	73	2.7	84.0

Units: nmol · min^{-1} · mg fraction protein^{-1}. MDH = malate dehydrogenase (matrix enzyme); Succ.cyt.c.red. = succinate cytochrome c reductase (inner membrane enzyme); MAO = monoamine oxidase (outer membrane enzyme).

subfractions and purified preparations of Complexes I, III, IV and V (tracks 4–8). Purified preparation of bovine creatine kinase and pyruvate dehydrogenase (Sigma, UK) did not cross-react with the patient's serum, thereby excluding them as possible candidates for the matrix antigen.

DISCUSSION

A total of 4 patients with Complex I defects and selective deficiency of the 24 kDa FeS protein have been described (Morgan-Hughes *et al.*, 1988; Schapira *et al.*, 1988). Two patients had myopathy alone, the other 2, including the patient described here, had encephalomyopathy. These observations indicate that 24 kDa deficiency is not associated with any single clinical syndrome and the variation in tissue expression may reflect the presence of tissue-specific isoforms of Complex I. Tissue-specific isoenzymes of Complexes I and IV have already been demonstrated in rat tissues (Kuhn-Nentwig and Kadenbach, 1985; Clay and Ragan, 1988).

The many possible molecular mechanisms underlying 24 kDa and Complex I deficiency have been discussed previously (Schapira *et al.*, 1988) but may involve defects in synthesis, transport from cell cytosol into mitochondria, or assembly of the Complex I holoenzyme. The deficiency of subunit II in Complex IV probably accounts for the low level of cytochrome aa_3, as these components are in close structural association in Complex IV. The normal oxidation rates utilizing both succinate and ascorbate + TMPD would indicate that Complex IV was not limiting the rate of NAD-linked substrate oxidation. Deficiencies of both the nuclearly encoded 24 kDa FeS protein and the mitochondrially encoded subunit of Complex IV could be due to defects in both genomes or could involve a disturbance of intergenomic regulation. Combined deficiencies of mitochondrially and nuclearly encoded polypeptides in Complexes I and IV have been reported in a hamster lung mutant cell line (Malczewski and Whitfield, 1984), but the precise molecular mechanism of this bigenomic defect remains uncertain.

The ragged red fibres seen on muscle biopsy represent an increase in mitochondrial number in certain fibres. Immunoblotting with PBC antibody has confirmed that there was an increase in mitochondrial protein in E.C. per gram of muscle. This may represent a 'compensatory hypertrophy' in response to the underlying biochemical defect. Despite an overall increase in total mitochondrial protein, however, the immunoblots showed a deficiency of Complex I not only per gram of mitochondria but also per gram of muscle homogenate. The observed increase in mitochondria numbers therefore does not appear to compensate for the biochemical defect at a functional or structural level.

Whether this case is sporadic or whether the patient's mother has a milder form of the same disease is unclear. Although the mother had suffered seizures when aged 25 yrs, she had had none for several years. Although she was on anticonvulsants when the patient was conceived, there is no known relationship between such medication and the development of respiratory chain defects. There was no clinical evidence of any mitochondrial disorder and although a biopsy from peroneus brevis showed ragged red fibres, control data from this muscle are limited. Deletions of mtDNA were not found in the patient's muscle (Holt *et al.*, 1988), but the mother's biopsy was not examined for deletions.

The serum of the patient in this report contained a circulating antibody to a single basic mitochondrial polypeptide of apparent molecular mass 41 kDa. All 13 products encoded by mtDNA are constituents of the respiratory chain and H^+-ATPase. The target polypeptide must therefore be nuclearly encoded as it was not present in purified preparations of Complexes, I, III, IV or V. The polypeptide was found in heart, skeletal muscle, liver, kidney and brain mitochondria from human, bovine, rabbit and rat sources and was isolated to the matrix fraction of rat liver mitochondria. Conservation of the polypeptide's molecular mass and antigenicity in all tissues and species examined suggests that it may play an important role in mitochondrial function.

Skoglund (1979) reported a patient with very similar clinical characteristics to the case described here. An autoantibody screen showed Skoglund's patient to be rheumatoid factor and antimitochondrial antibody positive. The characteristics of the mitochondrial antibody were not identified. The patient responded dramatically to prednisone and any attempt at dose reduction led to relapse.

The relevance of the circulating antibody to the pathogenesis of the patient's biochemical defect is unclear. Antibodies to a mitochondrial matrix protein could arise through cross-reaction with some external (e.g., viral) protein sharing a common antigenic epitope, or through the selective leakage of the 41 kDa protein from damaged mitochondria. Certain antibodies can gain access to the cell cytoplasm and produce tissue damage (Alarcon-Segovia *et al.*, 1978; Mathews and Bernstein, 1983). It is not known whether antibodies can penetrate mitochondrial membranes. As the 41 kDa antigen is a non-respiratory chain protein, it must be a nuclearly encoded and cytoplasmically translated polypeptide. Therefore, an antibody to a matrix protein could have access to its antigen during the transport of the protein from cell cytoplasm to mitochondrion. Matrix proteins (e.g., proteases) are necessary for the processing of some nuclearly encoded respiratory chain polypeptides whilst others (e.g., chaperonins) are thought to be involved in their assembly into functioning holoenzymes (Hemmingsen *et al.*, 1988; Pfanner *et al.*, 1988). Antibodies directed against such proteins could lead to a selective respiratory chain defect if they had access to the antigen. Multisystem failure in this case may be explained by the ubiquity of the 41 kDa protein. Organs most dependent on aerobic metabolism for energy would be those most susceptible to damage.

The treatment of patients with mitochondrial myopathy has so far proved rather disappointing (Przyrembel, 1987). Whether a subgroup of patients with multisystem mitochondrial disease due to the presence of an autoimmune response to specific mitochondrial proteins exists must await further studies. However, if such a group emerges and the target antigens are identified then a trial of immunosuppression in these patients would be justified.

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ACUTE INFANTILE SPINAL MUSCULAR ATROPHY

MUSCLE APOPTOSIS AS A PROPOSED PATHOGENETIC MECHANISM

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SUMMARY

Biopsy as well as autopsy studies of a child who died 8 weeks after birth from the acute infantile form of spinal muscular atrophy revealed classical morphological changes, including degeneration and loss of motoneurons in the spinal cord, loss of large myelinated fibres in anterior roots and neurogenic atrophy in muscle. New ultrastructural findings include massive muscle cell elimination by apoptosis with the formation of membrane-bound muscle cell fragments, apoptotic bodies. In addition, numerous immature muscle fibres were observed. The morphological findings raise the possibility that in a severely growth-retarded muscle, the process of muscle cell apoptosis removes the peripheral target of anterior horn cells resulting in secondary motoneuron death.

INTRODUCTION

Spinal muscular atrophy (SMA) is a degenerative disease predominantly of the lower motor neurons. Almost all reported cases of infantile SMA with death before 1 yr of age had prominent loss of the lower motor neurons (Byers and Banker, 1961; Emery, 1971; Chou and Nonaka, 1978; Towfighi *et al.*, 1985). The younger the diseased infant the more pronounced and more widespread are the morphological changes. We report a fatal case of SMA in whom we observed massive elimination of muscle cells by apoptosis during the neonatal period. Since the death of muscle cells by apoptosis has never been demonstrated in infants with SMA, this finding may shed some light on the pathogenetic mechanisms in this disease.

CASE REPORT

The female infant (I.B.) was born at term. The pregnancy was unremarkable except for decreased fetal movements during the final 2 months. At birth, the infant was inactive, hypotonic, apnoeic and required intubation and ventilation. The Apgar scores were 3, 7 and 8 at 1, 5 and 10 min, respectively. Physical examination disclosed an alert infant with very poor muscle development, generalized severe muscle weakness, fasciculation of the tongue and absent tendon reflexes. Contractures at the elbows and knees were noted. Because of cardiac insufficiency, echocardiography was undertaken and revealed a ventricular septal defect with a persisting ductus arteriosus. At the age of 6 wks, muscle as well as sural nerve biopsies were performed. The patient deteriorated neurologically. The usual therapeutic procedures were unsuccessful and at the age of 8 wks she died from cardiorespiratory insufficiency.

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MATERIAL AND METHODS

Biopsied tissues

Quadriceps femoris muscle and sural nerve were studied by light and electron microscopy. Serial frozen muscle sections for light microscopy were stained with haematoxylin and eosin (HE), modified Gomori trichrome, NADH-TR, menadione-linked alpha-glycerophosphate dehydrogenase (MAG), oil red O, PAS, myophosphorylase, acid phosphatase, ATPase after preincubation at alkaline (9.4) and acid (4.5 and 4.3) pH. For electron microscopy, specimens of both the muscle and the nerve were fixed in glutaraldehyde in phosphate buffer and postfixed in 1% osmium tetroxide in the same buffer. They were dehydrated and embedded in epoxy resin. Epon sections were stained with methylene blue for light microscopy. Thin sections double-stained with uranyl acetate and lead citrate were examined with a Philips 301 electron microscope.

Autopsied tissue

This included paraffin sections from the spinal cord, anterior and posterior roots and muscle (quadriceps femoris, deltoid, biceps brachii, triceps surae, intercostals and diaphragm). In addition, samples from quadriceps femoris fixed in formalin were examined by electron microscopy and the findings were compared with the ultrastructural features of the same muscle biopsied previously.

RESULTS

Biopsy findings

Morphological analysis of the quadriceps muscle showed a great predominance of small muscle fibres (fig. 1A) with a few of normal or enlarged diameter. There were central nuclei in 30% of the fibres. No inflammatory changes were observed. Numerous

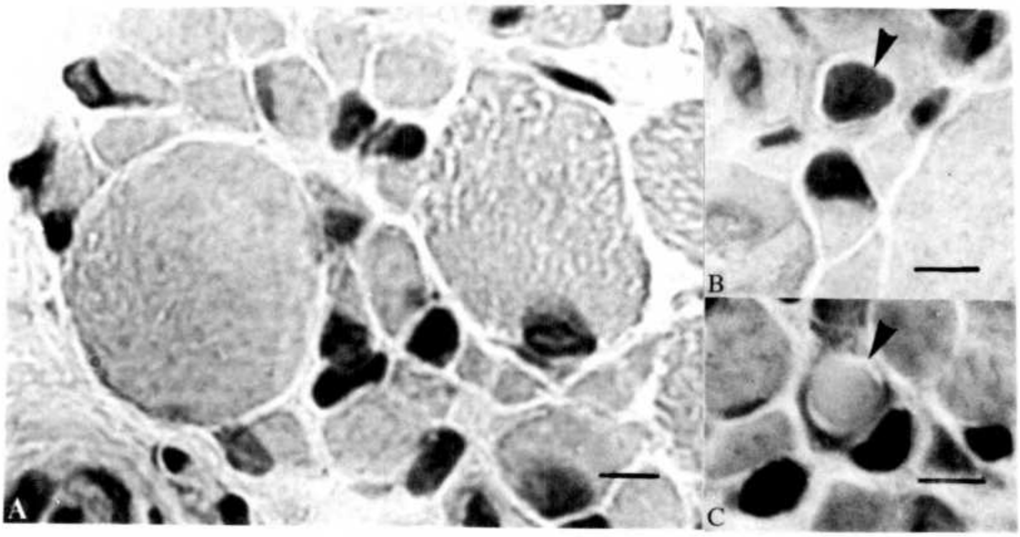

FIG. 1. Quadriceps femoris muscle. A, showing denervation changes with a collection of small muscle fibres. Haematoxylin and eosin. Bar = 50 μm. B, basophilic inclusion (arrow) surrounded by a clear halo. Bar = 75 μm. C, eosinophilic inclusion also surrounded by a clear halo (arrow). Bar = 75 μm.

spherical or ovoid bodies scattered in muscle cells were present. They varied considerably in size and number. Many appeared acidophilic (fig. 1C), some were basophilic (fig. 1B). They were negative with PAS, oil red O, acid phosphatase and modified trichrome stains. Electron microscopy revealed three distinctly different cell types in the muscle: (1) muscle cells of normal diameter with well organized sarcoplasm and peripherally located nuclei; (2) muscle cells that we have termed 'resting immature cells' which were very small, round or oval, with a single heterochromatic nucleus, centrally or peripherally located, and satellite cells (fig. 2A); (3) muscle cells with the appearance of active immature cells, some of which were arranged in clusters (fig. 3). In each cluster, 2 or 3 cells in different stages of maturation were closely connected and enveloped by a common basal lamina. Myotube-like cells with enlarged euchromatic central nuclei and large distinct nucleoli were frequently observed (fig. 2B). Their cytoplasm contained a large number of polyribosomes. Some filaments were arranged in hexagonal arrays. Among the muscle fibres described above there were a great number of single necrotic fibres. The morphology of these fibres was highly characteristic. The nuclear outline was abnormally convoluted and the nuclear chromatin was aggregated in dense masses (fig. 4). Myofibrils and sarcoplasmic organelles were compacted and very dense. In some instances, the sarcoplasm of necrotic fibres was composed of amorphous masses without any sarcomere pattern. Apart from the necrotic muscle fibres, numerous cell fragments were observed in the cytoplasm of healthy fibres. Cellular fragments appeared as large round or oval membrane-bound bodies, identifiable as apoptotic bodies (ABs). Their number, size and composition varied widely. Some contained nuclear fragments

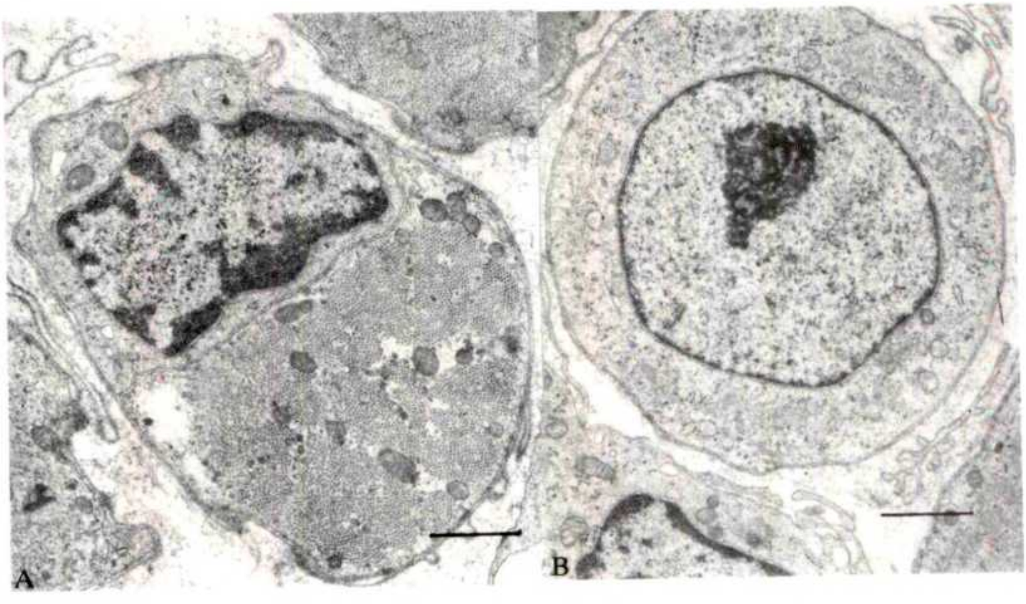

FIG. 2. A, immature fibre with satellite cell. Bar = 1.4 μm. B, myotube-like cell. Bar = 1.4 μm.

aggregated in dense masses (fig. 5A), others harboured only sarcoplasmic elements. The large ABs frequently contained structurally intact myofibrils (fig. 5B), the smaller ones partially degraded myofibrils and mitochondria with focal flocculent densities (fig. 6A). Several bodies contained fibrillar or amorphous electron dense material (fig. 6B). Most ABs were located deep within the cytoplasm of immature as well as mature muscle fibres. Other more peripherally located ABs were surrounded by cytoplasm of adjacent cells. Figure 7 illustrates fibrillar remnants located between immature cells lying within a common basal lamina. Remnants of degraded ABs were also observed within the cytoplasm of immature muscle fibres. Intramuscular nerves as well as the sural nerves showed a great number of unmyelinated fibres, some of which were arranged in multiaxonal bundles within a single Schwann cell (fig. 8A). There were myelinated fibres whose myelin sheaths were too thin in relation to their axonal diameters; other fibres showed wallerian degeneration. Bands of Büngner devoid of axons were also found (fig. 8B).

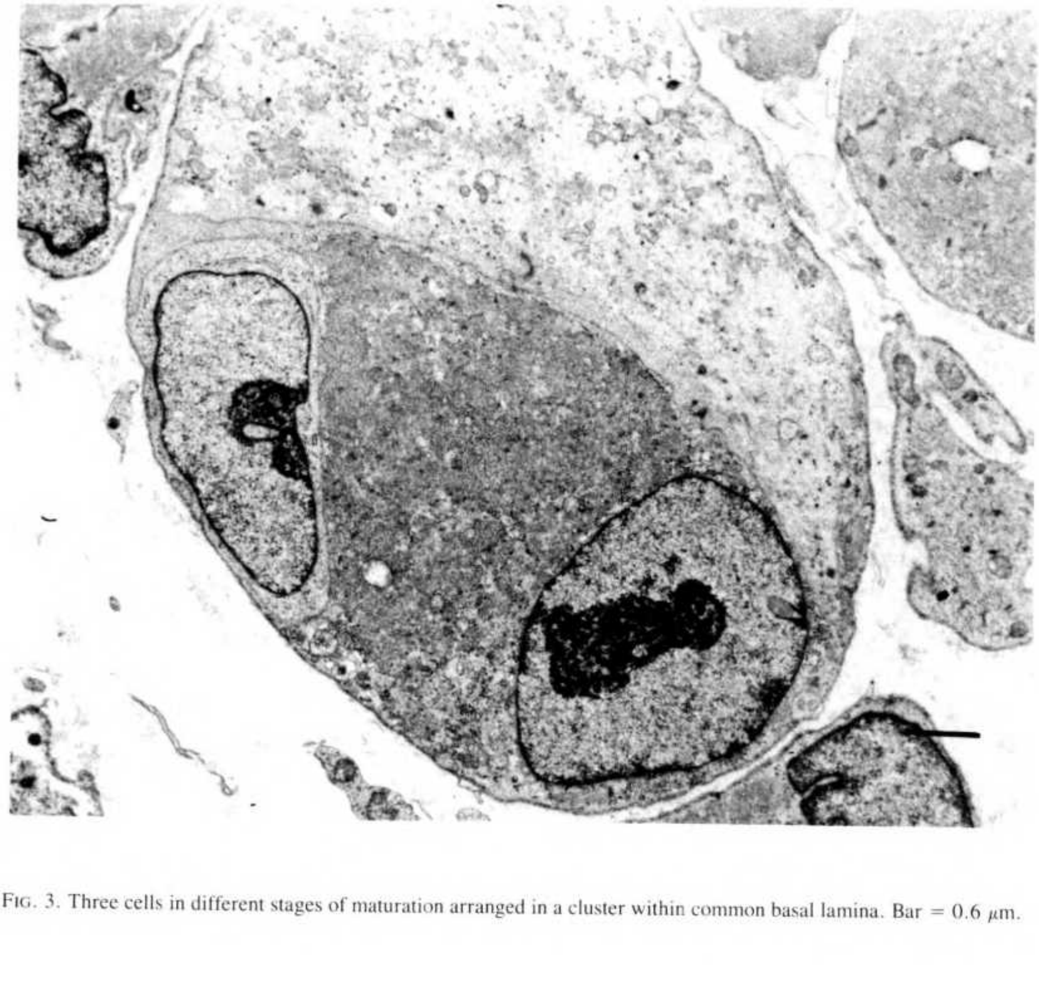

FIG. 3. Three cells in different stages of maturation arranged in a cluster within common basal lamina. Bar = 0.6 μm.

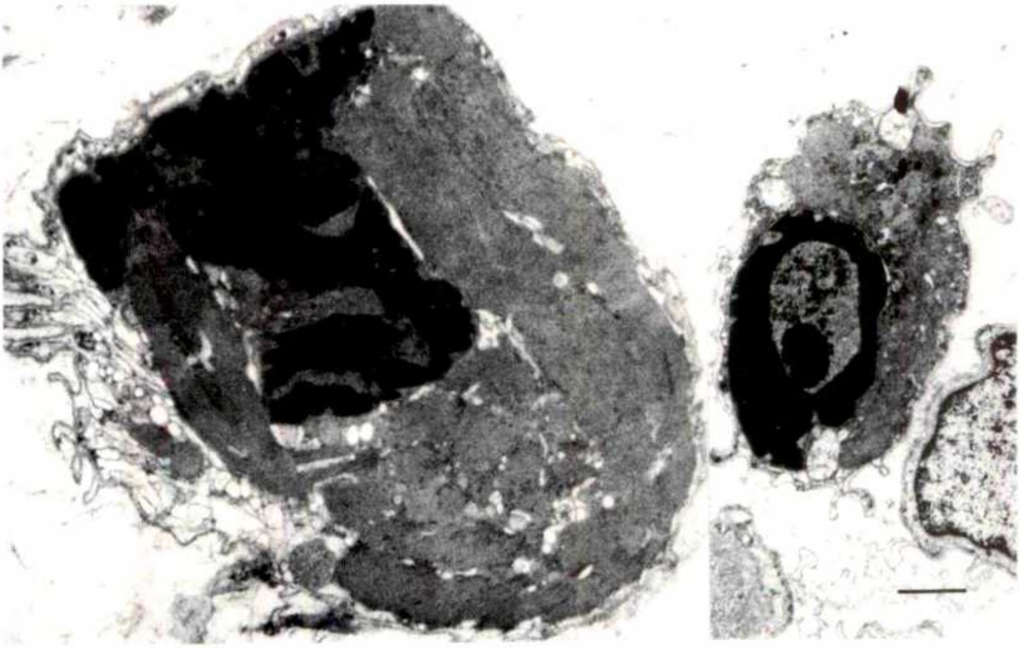

FIG. 4. Apoptotic muscle cells. The sarcoplasm is condensed and homogeneous. Chromatin is aggregated in a large compact granular mass. Bar = 1.1 μm.

Postmortem examination

The spinal cord showed severe neuronal loss in the anterior horns at all levels (fig. 9A, B). At the lumbosacral level, ventral clusters of motoneurons were unremarkable (fig. 9B), the remaining few laterally located motoneurons were atrophic close to empty cell beds. Occasional neurons displayed chromatolysis. Both anterior and posterior roots showed loss of myelinated axons with the anterior roots being more severely involved. No glial bundles were observed in the spinal roots. Skeletal muscle sections from quadriceps femoris, biceps brachii, deltoid, triceps surae and the intercostal muscles showed pronounced atrophy (fig. 10). The number of muscle fibres with normal diameters in the quadriceps had diminished considerably as compared with the preceding biopsy specimen. There were only a few muscle fibres with a normal diameter. By electron microscopy, quadriceps muscle fibres were much smaller and contained fewer myofibrils in comparison with those of the biopsy specimen. Immature activated muscle fibres seen in the biopsied muscle as well as apoptotic bodies were not found. All small muscle fibres had a reduced number of myofibrils, resulting in distinct rarefaction of the sarcomeres. Although these degenerative changes gave rise to sarcolemmal tubes which were almost completely devoid of myofibrils, no accumulation of nuclei was observed.

DISCUSSION

In the patient presented here, generalized muscle weakness, atrophy and fasciculation, loss of anterior horn cells, and a rapidly progressive clinical course leading to death

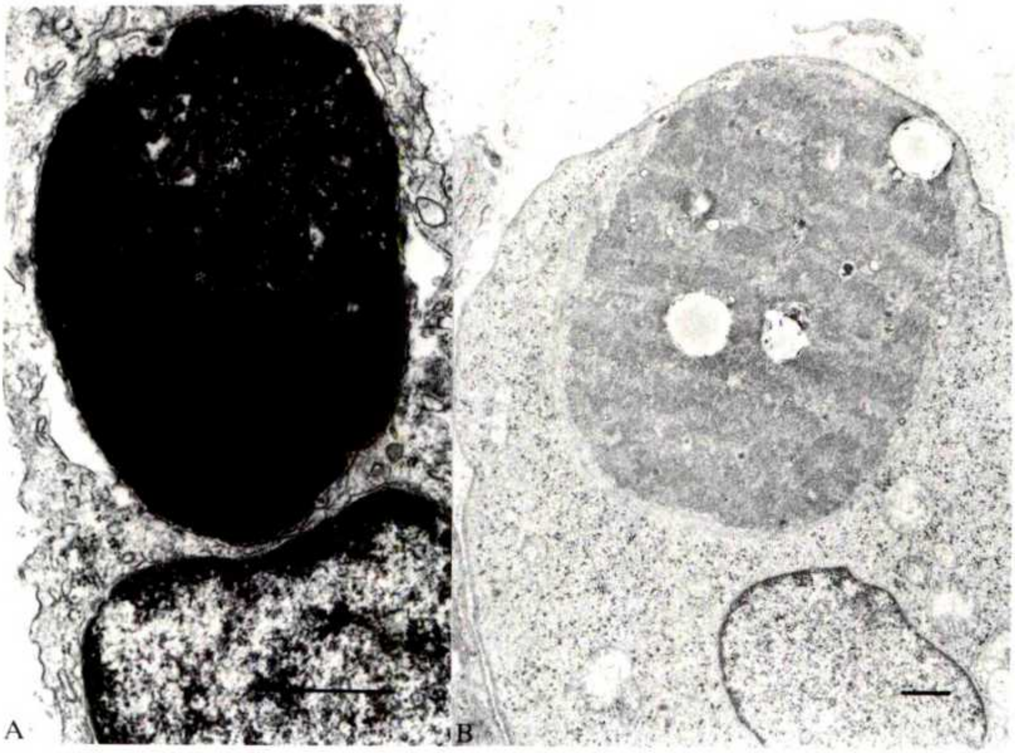

FIG. 5. A, apoptotic body containing a recognizable nuclear fragment. Bar = 2.3 μm. B, apoptotic body with fragments of sarcoplasm and myofibrils. Bar = 0.8 μm.

before the age of 1 yr, permit a diagnosis of the acute infantile form of SMA. In addition to the classical morphological findings such as degeneration and loss of motoneurons in the spinal cord and a neurogenic pattern of muscle pathology, our study presents new findings. These indicate that in severely growth-retarded muscle, numerous cells undergo elimination by apoptosis. This is manifested by the appearance of acidophilic or basophilic inclusions within muscle fibres. Structural analysis disclosed the presence of numerous necrobiotic cells and formation of a number of membrane-bound muscle fibre fragments with relatively well preserved organelles. They had been degraded by neighbouring immature muscle cells.

All these morphological changes observed in the muscles of our patient were the same as those described in the process of apoptosis. The term apoptosis was proposed by Kerr *et al.* (1972) for cell death which appears to play a complementary role in the regulation of animal cell populations. In the last decade it has been shown that there are two distinct mechanisms of cell death (Wyllie *et al.*, 1980). The first is the long established process of 'coagulative' necrosis which normally involves groups of cells affected by adverse conditions. The second mechanism has been termed apoptosis. It affects individual cells and has been shown in certain cases to be under physiological

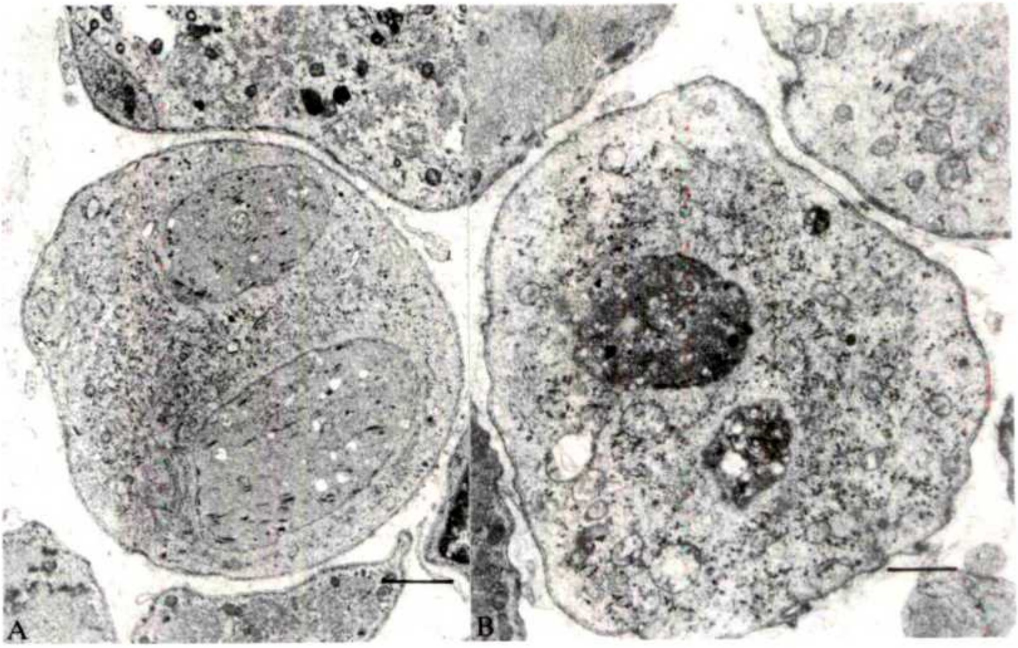

FIG. 6. A. 2 apoptotic bodies within an immature muscle cell. Note flocculent densities. Bar = 1.1 μm. B, dense residual bodies. Bar = 1.1 μm.

control (Kerr *et al.*, 1972; Wyllie *et al.*, 1980). To date, the most efficient way of differentiating between cells undergoing necrosis and apoptosis is based on the identification of specific morphological changes seen at the ultrastructural level (Wyllie *et al.*, 1980). Apoptosis is characterized by nuclear and cytoplasmic condensation and exuberant budding to produce small membrane-bound compact cell fragments. These are rapidly ingested by adjacent intact cells and undergo changes that resemble coagulative necrosis. Apoptosis is responsible for the focal elimination of cells during embryonic development (Kerr *et al.*, 1972; Wyllie *et al.*, 1980; Wyllie, 1987) and metamorphosis (Kerr *et al.*, 1974; Lockshin, 1981). Muscle cell elimination in the tadpole tail during spontaneous metamorphosis is characterized by condensation of nuclear chromatin, break up of nuclei and cytoplasm, and the formation of membrane-bound fragments, approximately 5–20 sarcomeres in length, termed 'sarcolytes'. The structural pattern of sarcolytes appears to be identical to that observed in our study. Fragmentation of dead cells is followed by degradation of remnants within neighbouring cells and macrophages. A similar process of apoptosis has been described by Hurle *et al.* (1977) in embryonic heart and in smooth muscle in neonatal rats (Hoyes and Barber, 1983). Webb (1972, 1977) studied muscle cell death in human embryos. Combining electron microscopy with a range of histochemical tests, he reported that muscle cell death was found in all fetuses between 10 and 16 wks of gestation. They suggested that disintegration and disruption of the cell membrane were crucial factors. All these findings indicate

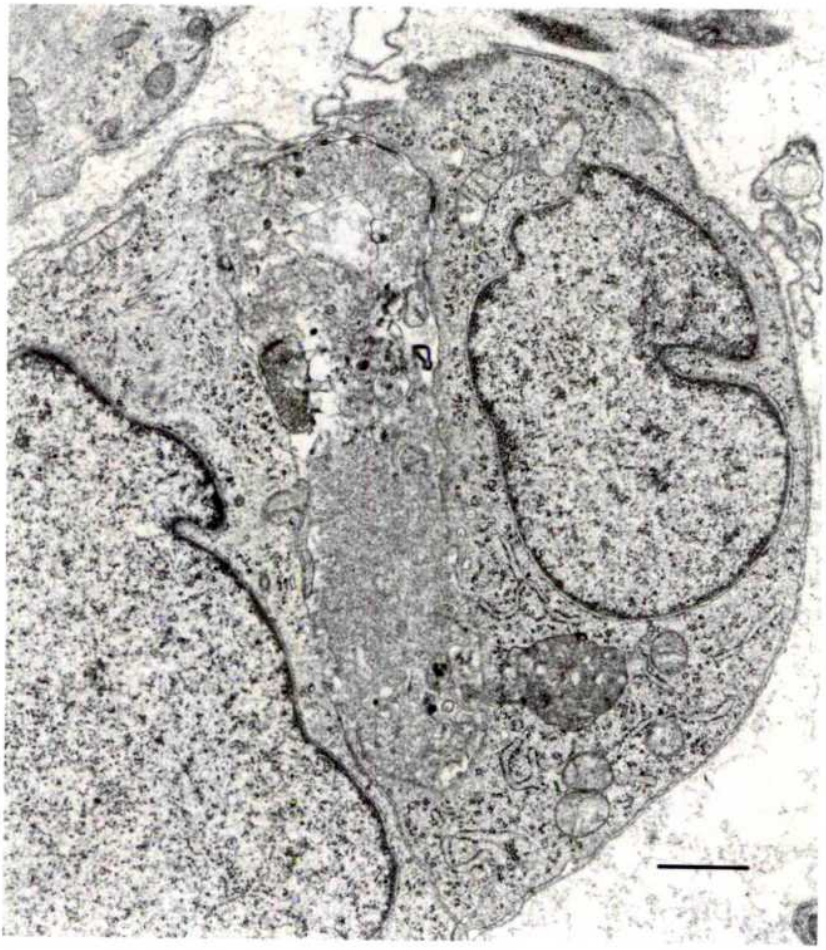

FIG. 7. Fibrillar remnants located between immature cells lying beneath a common basal lamina. Bar = 1.1 μm.

that cell death is indeed a normal part of the morphogenetic or developmental sequence, and that the eliminated tissue or organs are useful only during embryonic or larval life.

The fact that muscle cell death by apoptosis occurs in acute infantile SMA is an intriguing finding. The fetal character of numerous muscle fibres seen in our child is a characteristic and common finding that has been observed in several cases of SMA (Fidziańska, 1974, 1976; Hausmanowa-Petrusewicz and Fidziańska, 1974; Fidziańska and Hausmanowa-Petrusewicz, 1984). The ultrastructural appearance of small muscle fibres with the morphology of myotubes, as well as immature muscle fibres arranged in clusters, resembles that of muscle fibres seen at 16 wks of fetal life (Fidziańska, 1980) and suggests failure in muscle maturation. Comparing myotube-like cells, as well as immature fibres arranged in clusters, to those previously described (Fidziańska and Hausmanowa-Petrusewicz, 1984), two differences were recognized in our patient's

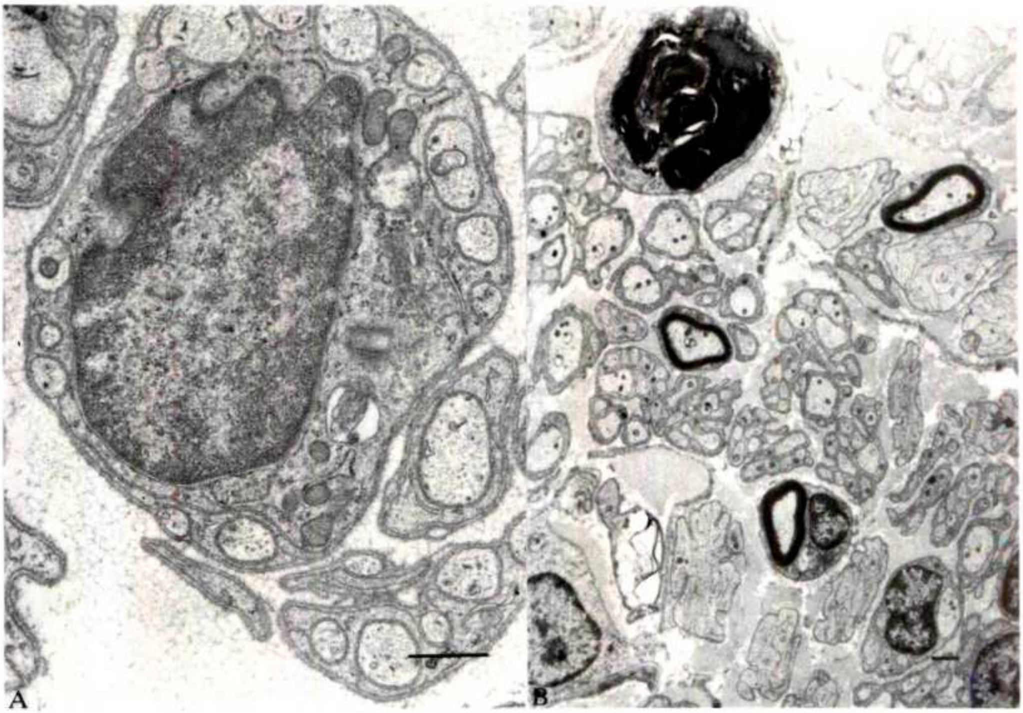

FIG. 8. A, part of an intramuscular nerve. Several axons are surrounded by the cytoplasm of a single Schwann cell. Bar = 1.4 μm. B, sural nerve showing a number of unmyelinated fibres. Note one fibre in upper part of figure undergoing wallerian degeneration. Bar = 0.4 μm.

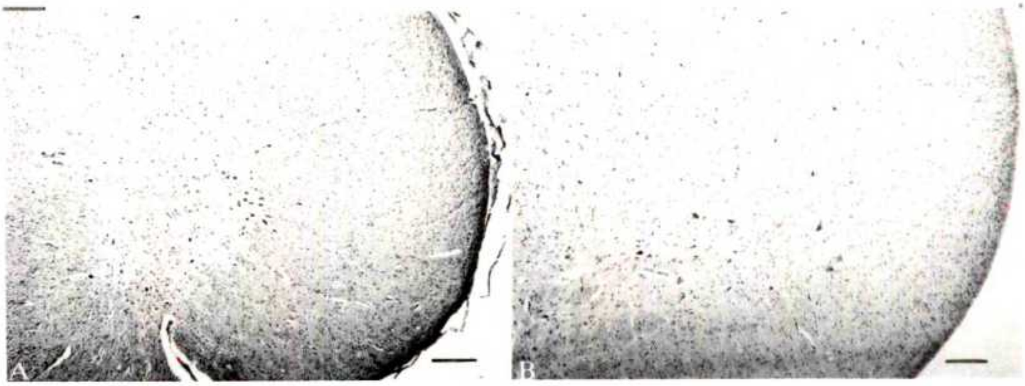

FIG. 9. A, spinal cord, cervical level, showing a striking loss of large anterior horn cells. Bar = 100 μm. B, lumbosacral level showing a pronounced reduction of neurons. Bar = 100 μm.

muscle. First, immature muscle cells were much more active metabolically than those described earlier and, secondly, they contained numerous ABs which have never been observed before. Metabolically active immature cells in our study possessed enlarged

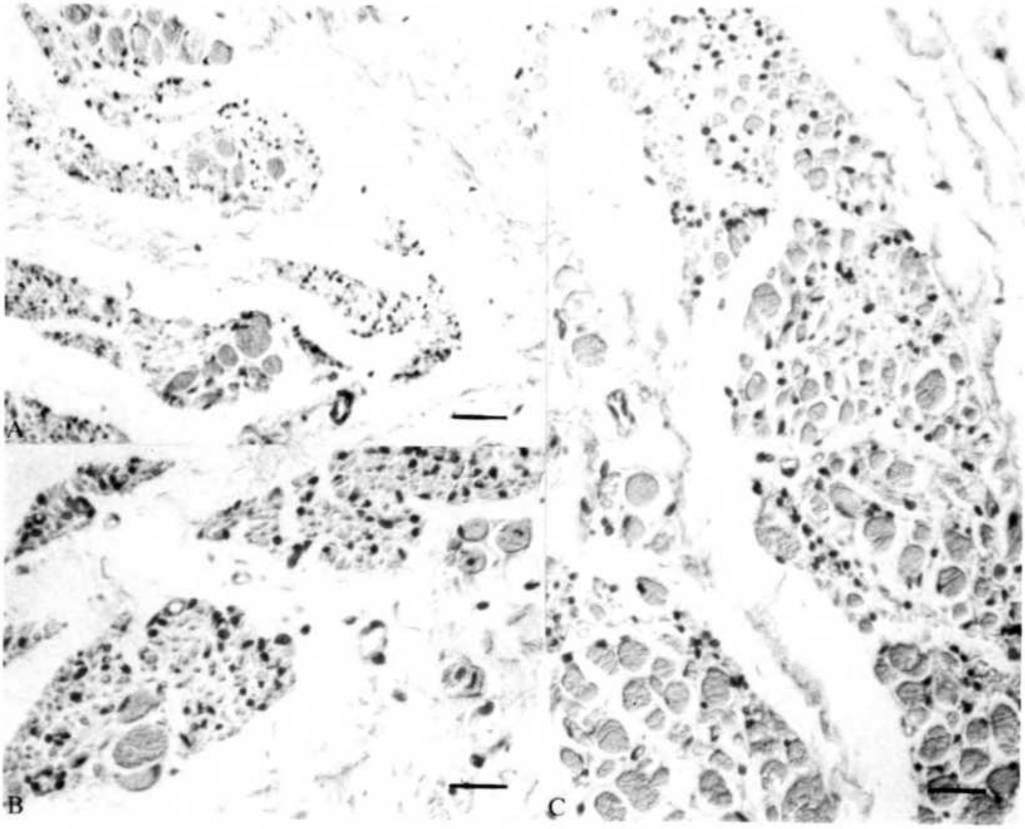

FIG. 10. Autopsied muscles showing pronounced atrophy. Haematoxylin and eosin. Bar = 20 μm. A, biceps brachii; B, quadriceps femoris; C, intercostal muscle.

euchromatic nuclei and prominent enlarged nucleoli. However, enlargement of the nucleolus is a hallmark of malignancy; large nuclei are also a common finding in proliferating cells, as well as in cells producing protein-rich products (Ghadially, 1982). Activity of immature cells coincides with the appearance of ABs within their cytoplasm.

The question arises as to how ABs can come to lie under the basal lamina of cell clusters. A possible explanation is that a single cell lying inside a cluster undergoes necrosis and is taken up by its neighbours and is then digested. Phagocytic activity by striated muscle fibres has been demonstrated in culture (Garfield *et al.*, 1975), in embryonic heart (Hurle *et al.*, 1977), and during metamorphosis (Kerr *et al.*, 1974). During human myogenesis, muscle cells which degenerate do so at the myotube stage (Webb, 1972, 1977). In recent studies (Fidziańska and Goebel, unpublished) we have found that naturally occurring muscle cell death appears as a successive two-stage wave of cell necrosis. The first is observed at the myotube stage when a number of muscle cells are eliminated. Later, at 20 wks of gestation, a single cell is removed from a cluster

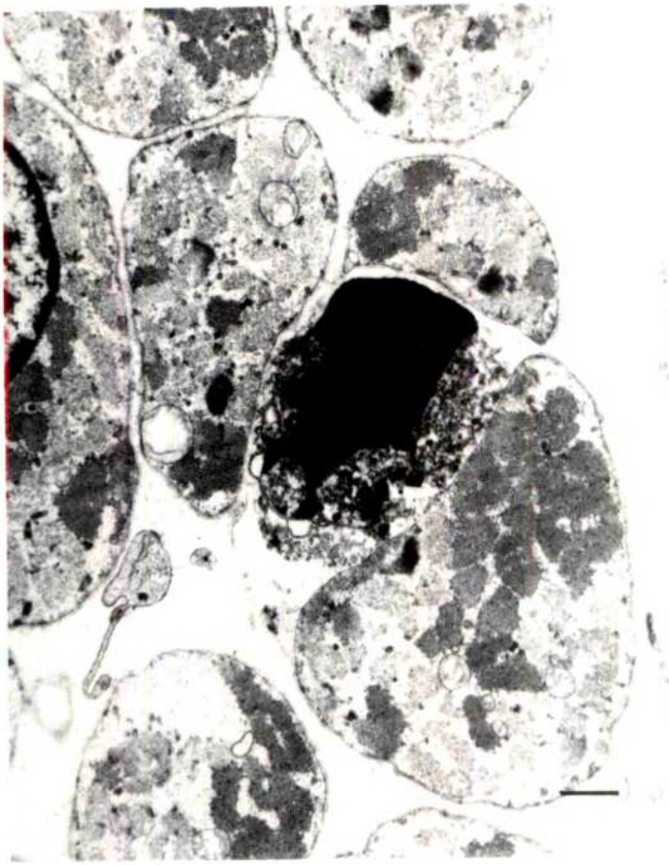

FIG. 11. Quadriceps femoris from a 10-wk-old human fetus. One cell in a cluster which forms an immature muscle fibre is undergoing apoptosis. Bar = 0.9 μm.

(fig. 11). This seems to be responsible for final muscle fibre size and shape. If the process of muscle cell death is more extensive or repeated later than normal, that is, at 20 wks, the formation of muscle fibres may be severely affected. All presented findings allow us to speculate that in growth-retarded muscle, the process of muscle apoptosis may also be prolonged or repeated. This could result in protracted muscle cell death which may lead to a greater reduction in the number of muscle fibres and therefore in the final size of the muscle (fig. 12). Removal of the peripheral targets of anterior horn cells results in secondary death of motoneurons. It is now well documented that the final number of motoneurons in the spinal cord following fetal development depends on the input from muscle for survival (Hollyday and Hamburger, 1976; Prestige, 1976; Lamb, 1981). Naturally occurring death of motoneurons is accentuated by the removal of the target muscle (Okado and Oppenheim, 1984). The temporal relationships seen in our patient in muscle, together with peripheral nerve immaturity and muscle apoptosis,

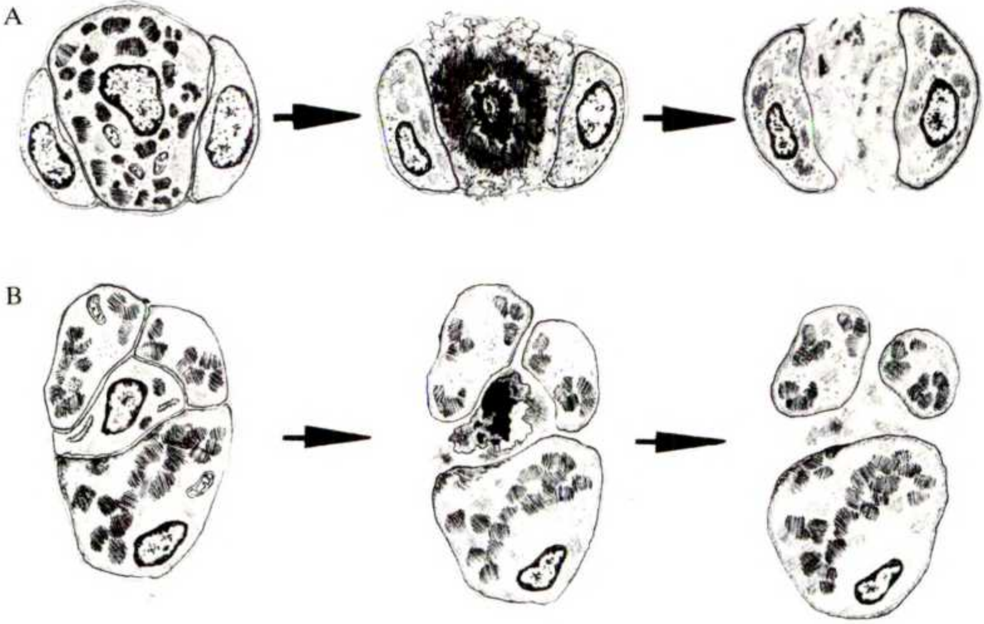

FIG. 12. Schematic diagram of the authors' concept of, A, muscle cell elimination by apoptosis at the myotube stage, and B, at the stage of immature muscle fibres.

and degeneration and disappearance of anterior horn cells, makes our interpretation more realistic.

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PERIPHERAL NEUROPATHY IN LEIGH'S DISEASE

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SUMMARY

Sural nerves were examined in 3 childhood cases of Leigh's disease (from 2 families), each with electrophysiological documentation of peripheral neuropathy. Postmortem confirmation of Leigh's disease was made in 2 cases; the third had characteristic CT scan appearances. Myelinated fibre densities were within normal limits, with no evidence of degeneration or regeneration. The myelin sheaths, however, appeared thin and this was confirmed quantitatively. In teased fibres there was no demyelination in 2 cases but some in 1 case. The findings suggest that there is hypomyelination of peripheral nerves leading eventually to demyelination.

INTRODUCTION

Leigh's subacute necrotizing encephalomyelopathy (Leigh, 1951) can no longer be regarded as a single nosological entity. Despite clearly defined neuropathological features, there is a wide range of clinical presentations. The recent demonstration of mitochondrial cytopathy (Crosby and Chou, 1974; Egger *et al.*, 1982) and a variety of enzyme defects, namely cytochrome c oxidase (Willems *et al.*, 1977; Miyabayashi *et al.*, 1985; Lake and Harding, 1987), pyruvate decarboxylase (Evans, 1981; Miyabayashi *et al.*, 1985), pyruvate dehydrogenase (De Vivo *et al.*, 1979; Kretzschmar *et al.*, 1987) and pyruvate carboxylase (Gilbert *et al.*, 1983) have led some workers to the concept that Leigh type lesions are the common end result of various defects of the respiratory chain (Walter, 1983; Lake and Harding, 1987).

Since Leigh's disease may be part of a multisystem disorder including mitochondrial myopathy with ragged red fibres (Crosby and Chou, 1974; Egger *et al.*, 1981, 1982), it is perhaps surprising that there are relatively few reports of nerve conduction studies or detailed morphological observations on peripheral nerve in the literature. At present there is only one study that includes morphometry. Goebel *et al.* (1986) provided evidence, in 4 cases, of thinly myelinated peripheral nerve fibres, but their methodology did not allow further explanation of this finding.

We report peripheral nerve pathology with full quantification in 3 children, all with detailed electrophysiological documentation of peripheral neuropathy. The diagnosis of Leigh's disease was verified at autopsy in 2 cases, while the third, a sibling of the first case, had characteristic CT scan appearances.

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CASE REPORTS

Case 1

R.P., female. First child of unrelated East African Asian parents. Walking delayed until 18 mos; gait wide based and unsteady. Progressive hirsutism from 9 mos; endocrine investigations negative. Possible encephalitic episode at 3½ yrs for which no cause was found. Gradual development of ataxia and ophthalmoplegia.

At 5 yrs, investigated at Hospital for Sick Children with her sister. Plasma pyruvate and lactate significantly elevated; other biochemistry, CT brain scan, EEG, ERG and VER normal. Nerve conduction studies: small delayed sensory and mixed action potentials; pronounced slowing of maximum motor nerve conduction velocity (MNCV), interpreted as a mixed (sensory-motor) demyelinating type of polyneuropathy (*see* Table 1). Muscle biopsy: increased variability of fibre diameters with sizes ranging from 17 to 50 μm (normal for age 22−23 μm). Scattered fibres with an increased lipid content; no ragged red fibres. Histochemical staining for mitochondrial enzymes revealed normal NADH dehydrogenase and alpha-glycerophosphate dehydrogenase activity; cytochrome c oxidase activity grossly reduced. Type 1 fibre predominance. The extent of the lipid deposition justified the description of a lipid storage myopathy.

At 9 yrs developed dysphagia, emotional incontinence and sleep disturbance. Height and weight on third centile, head circumference on fiftieth. Marked hirsutism, jerky nystagmus on lateral and upward gaze, limb and trunk ataxia, hypotonia, depressed tendon reflexes. CT scan: symmetric low density lesions in pallida and cerebellar atrophy.

Case 2

N.P. (sister of R.P.). Normal until rotavirus infection at 10 mos, when developmental progress tailed off. Became hirsute during first year. At 23 mos, biochemical and electrophysiological abnormalities similar to her sister (*see* Table 1). Normal CT scan.

At 3 yrs, increasing ataxia and tiredness; height and weight on third centile; marked hair growth on limbs, back and trunk; jerky nystagmus on lateral and upward gaze, intention tremor, broadbased gait, proximal muscle weakness and absent tendon reflexes. Skin fibroblast culture showed deficient cytochrome c oxidase activity and normal pyruvate dehydrogenase activity. Remained short and thin, gradually regressed; developed facial and later generalized muscle wasting and weakness. Evidence of autonomic dysfunction with vomiting, night soiling and an unusual breathing pattern. Died suddenly at 6½ yrs. Quadriceps muscle biopsy: muscle fibre diameters within normal size range for age. No myopathic changes, no ragged red fibres and no excess lipid. Normal fibre type distribution. Histochemically demonstrated NADH-dehydrogenase activity normal but cytochrome c oxidase very low in comparison both with heart from this patient and with normal muscle from other patients. Quantitative assay confirmed the deficiency in muscle.

Case 3

C.B. male, younger child of nonconsanguinous East African Asians. Maternal family history of diabetes mellitus. Maternal uncle died at 14 yrs with unspecified neurological disorder.

Early development normal. Chest infection at 11 mos, followed by failure to thrive, increasing irritability, generalized weakness and virtual cessation of development. On examination at 26 mos, notably hirsute and very irritable; dilated pupils, poor light response, right convergent squint, intermittent rotatory nystagmus, and bilateral temporal pallor on fundoscopy. There was hypertonia with marked loss of muscle bulk but no obvious weakness. Tendon reflexes brisk and plantar responses extensor. CT scan: symmetric low densities involving the pallida and dentate nuclei. Needle muscle biopsy: type 2 fibre atrophy. No myopathic features, no ragged red fibres and no increase in lipid. Mitochondrial enzyme activity (NADH-dehydrogenase and succinate dehydrogenase) normal but cytochrome c oxidase activity lower than normal. Nerve conduction studies: sensory and mixed nerve action potentials small, dispersed and delayed, interpreted as a sensory-motor, probably demyelinating polyneuropathy (*see* Table 1). EEG, VER and ERG normal. Brainstem auditory evoked responses (BAER): absence of clearly defined later components suggesting abnormality in the upper brainstem.

Random, fasting and postglucose load lactates normal. Aspartate transaminase significantly elevated (97 U/l).

Death followed cardiac arrest at 2 yrs 9 mos.

METHODS

Peripheral nerves were removed postmortem from Cases 2 and 3 within 12 h of death. Sural nerves were taken from both cases, and the femoral nerve from Case 2; a biopsy was taken from Case 1. All sural nerves, including the controls, were taken from the retromalleolar region.

The nerves were fixed in 3% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.4; they were held under slight tension and allowed to adhere to pieces of card for about 15 s before immersion in fixative. Blocks of nerve for transverse sections were postfixed in osmium tetroxide and processed into Araldite. The remaining length of nerve was processed into unpolymerized Araldite for teasing. Semithin sections were stained with Azure II, basic fuchsin and methylene blue (Humphrey and Pittman, 1974), and ultrathin sections were stained with uranyl acetate and lead citrate.

Control data were taken from a previous quantitative study of sural nerves taken postmortem from subjects with no history of peripheral nerve disease (Jacobs and Love, 1985). In the present study, data from nerves of 3 and 5 yr-old children were used.

Quantitative measurements

Densities of myelinated fibres. Myelinated fibres were counted at the microscope with the aid of an eyepiece graticule. At least 1200 fibres were counted in 3–4 fascicles in each nerve. The area of the counted fascicles, and the total fascicular area were measured using a microscope with a drawing tube attachment extended over the bit-pad of a Kontron MOP AMO-3 image analyser. A LED on the MOP cursor could be seen superimposed on the image in the microscope, and the circumference of each fascicle was traced. Total numbers of myelinated fibres and their densities were calculated.

Myelinated fibre and axon diameters. Representative fields from all nerves were photographed. From prints enlarged to ×2880, the areas of fibres (outer perimeter of myelin sheath) and of the axon (inner perimeter of myelin sheath) were measured in about 400 fibres in each nerve, using the Kontron image analyser interfaced to the computer. Fibre and axon diameters, g ratios (axon diameter/fibre diameter) and myelin sheath thickness were computed assuming axonal circularity.

Myelin lamellae. Counts of myelin lamellae and axon diameters were made on a smaller sample (about 120 fibres) from the sural nerves of Case 2 and the 5 yr control. Electron micrographs at a final magnification of about ×9000 (calibrated with a grating replica) were used. Numbers of lamellae were counted using a binocular microscope; axon areas were measured using the Kontron MOP, and the diameter calculated assuming axonal circularity.

Regression lines, coefficients of correlation and the SE of regression were calculated for myelin sheath thickness or for numbers of myelin lamellae, relative to axon diameter. The regression lines were compared statistically (ANOVA).

Electron micrographs at a final magnification of approximately ×25 000 (calibrated with a grating replica) were used for measurement of myelin sheath thickness and for counting numbers of myelin lamellae.

Densities of unmyelinated axons. Unmyelinated axons were counted from electron micrographs enlarged to about ×10 000 (calibrated with a grating replica) in an area of approximately 0.01 mm^2.

Internodal lengths. Fibres were teased in Araldite on glass slides. Using the MOP image analyser as described above, sequential internodes of fibres were traced. The mean diameter of the thickest internode was calculated from 4 measurements made at points away from the Schwann cell nucleus, incisures or paranodes. Data were stored and analysed in an interfaced RML 380Z computer and displayed graphically as in the method of Fullerton *et al.* (1965).

RESULTS

Neuropathology

Case 2. The total fixed weight of the brain was 1125 g, that of the hindbrain being 110 g. The external appearance was normal. On coronal slicing there were symmetric circumscribed grey-brown foci in both subthalamic nuclei and the hypothalamus (fig. 1). In the cerebellum most of the central white matter in the vermis and hemispheres was soft and grey while throughout the brainstem there were small roughly symmetric grey lesions, clearly defined in the oculomotor nuclei, pontine tegmentum and linearly beneath the fourth ventricle, and irregularly defined in the medulla. The cervical and thoracic cord also showed central cruciate or linear grey lesions. Histologically, all these lesions had typical features of Leigh's disease, with loosening of the tissue, loss of myelin staining, marked capillary proliferation, gliosis and relative preservation of nerve cells (fig. 2).

Case 3. The total fixed weight of the brain was 1110 g, that of the hindbrain being 120 g. Externally it was normal. On coronal sectioning, symmetric red-brown discoloration and softening were present in both putamina, focally in the right globus pallidus, and in both subthalamic nuclei. The central white matter of the cerebellum was symmetrically affected, even necrotic. Bilateral lesions were also present throughout the brainstem and the cervical cord. Histology was typical of Leigh's disease.

Nerve conduction studies

Sensory and nerve action potentials (Table 1) were generally small or absent, with dispersed form and slowing of maximum conduction velocity by 10–30%, even when

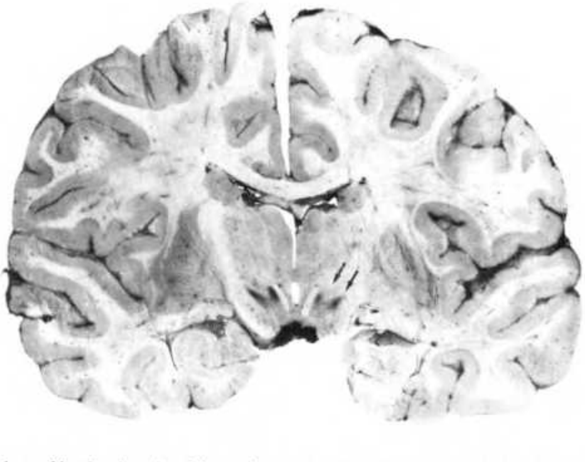

FIG. 1. *Case 2*. Coronal section of brain showing bilateral symmetric lesions in subthalamic and hypothalamic regions (arrows).

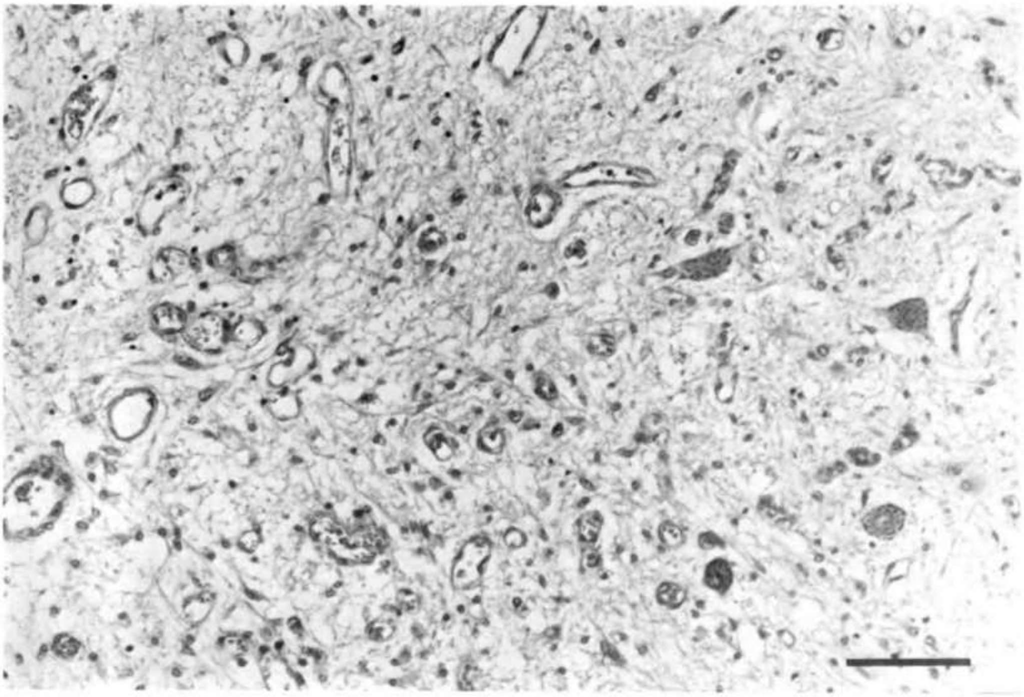

FIG. 2. *Case 2*. Histology of a typical lesion in the medulla showing prominent hypertrophic capillaries, loosening of the parenchyma and gliosis, but preservation of neurons. Bar = 100 μm.

TABLE 1. ELECTROPHYSIOLOGICAL RESULTS

	Case 1	*Case 2*	*Case 3*	*95% normal limits*
Sural nerve SAP	6 μV, 33 $m \cdot s^{-1}$	L absent R 4 μV, dispersed, 31 $m \cdot s^{-1}$	10 μV, dispersed 28 $m \cdot s^{-1}$	10 μV, 40 $m \cdot s^{-1}$
Median mixed NAP	ND	5 μV, bifid, 46 $m \cdot s^{-1}$	15 μV, dispersed 40 $m \cdot s^{-1}$	30 μV, 50 $m \cdot s^{-1}$
Abductor hallucis MAP				
Amplitude	ND	ND	4 mV	4 mV
Distal latency	ND	4.7 ms	4.0 ms	3.5 ms
F latency	54 ms**	ND	36 ms*	27*; 30**
Tibial MNCV	25 $m \cdot s^{-1}$**	19 $m \cdot s^{-1}$	ND	42 $m \cdot s^{-1}$*; 45 $m \cdot s^{-1}$**
Median MNCV	ND	31 $m \cdot s^{-1}$ with 50% decrement of proximal evoked potentials	ND	43 $m \cdot s^{-1}$
Tibialis anterior EMG	MUPs of up to 7 mV	Normal	Normal	2 mV

* Values for 3-yr-olds; ** Values for 5-yr-olds. ND = not done; MAP = muscle action potential; MUP = Voluntary motor unit potential; NAP = nerve action potential; SAP = sensory action potential; MNCV = motor nerve conduction velocity. Stimulation and recording were with surface electrodes; stimulus duration was 0.1 ms, rate 1/s.

amplitude was normal. Motor conduction velocity was more markedly slowed, by 30–50%, and F waves were proportionately delayed. In the single instance in which the evoked compound muscle action potential was compared on distal and proximal stimulation of the nerve, there was a 50% decrement in amplitude.

TABLE 2. DENSITIES OF MYELINATED AND UNMYELINATED AXONS

Case	Age (yrs)	Nerve	*Density/mm^2* Myelinated axons	Unmyelinated axons
1	5	Sural	9 981	79 989
2	5	Sural	10 583	64 000
2	5	Femoral	10 058	–
3	3	Sural	13 434	92 442
Control	3	Sural	12 940	87 200
Control	5	Sural	11 440	47 400

TABLE 3. REGRESSION OF MYELIN SHEATH THICKNESS ON AXON DIAMETER

Case	*Nerve*	*Slope*	*r*
1	Sural	0.077*	0.753
2	Sural	0.064*	0.584
2	Femoral	0.063*	0.665
3	Sural	0.095*	0.645
Controls			
3 yr	Sural	0.168	0.706
5 yr	Sural	0.196	0.880

* $P = < 0.0001$.

TABLE 4. REGRESSION OF NUMBERS OF MYELIN LAMELLAE ON AXON DIAMETER

Case	*Nerve*	*Slope*	*r*
1	Sural	5.48*	0.769
5-yr control		12.05	0.792

* $P = < 0.0001$.

Peripheral nerve studies

The myelinated fibre density was considered to be within the normal range for age in each case (Table 2) although the sural nerve from Case 1 was at the lower limit. In transverse sections, there was no evidence of fibre degeneration or of demyelination. However, after comparison with age-matched controls it was clear that myelinated fibres had thinner sheaths (fig. 3). This was confirmed quantitatively. Myelin sheath thicknesses were plotted against axon diameters, and the regression lines calculated (fig. 4, Table 3). In each case the slope relating these parameters was significantly ($P = <0.0001$) lower than the respective control. From electron micrographs of the biopsied nerve from Case 1, myelin lamellae were counted and axon diameters calculated (fig. 5). The slope of the regression line was again significantly different from the control nerve.

The g ratio was also calculated and plotted against axon diameter. Values for g were higher than those of controls (fig. 6), again confirming the thinness of the myelin sheaths. However, the use of g ratios as a means of showing changes in myelin thickness is

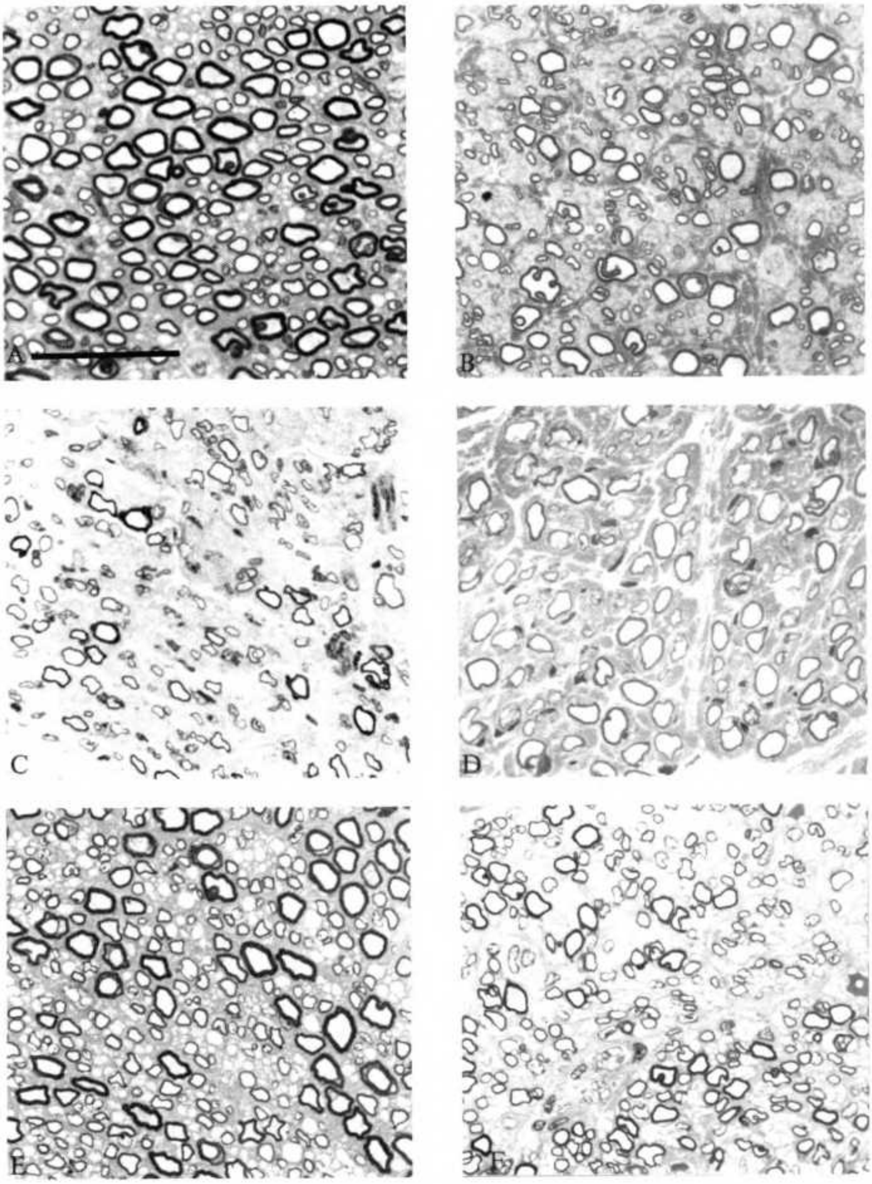

FIG. 3. Araldite sections (1 μm) all at same magnification. A, 5-yr-old control sural nerve. B, *Case 1*, sural nerve (biopsy); C, *Case 2*, sural nerve (PM); D, *Case 2*, femoral nerve (PM); E, 3-yr-old control sural nerve; F, *Case 3*, sural nerve (PM). Myelin sheaths of fibres in each of the patients' nerves are thinner than those of the respective control nerves. A few fibres are irregular in shape. Axons are thinner in *Case 3*, than in its control but sheaths are still relatively thin. Bar = 50 μm.

less convenient than the calculation of myelin sheath thickness and its regression with axon diameter, which can be assessed statistically.

Histograms of fibre diameters showed a bimodal distribution in the sural nerve of

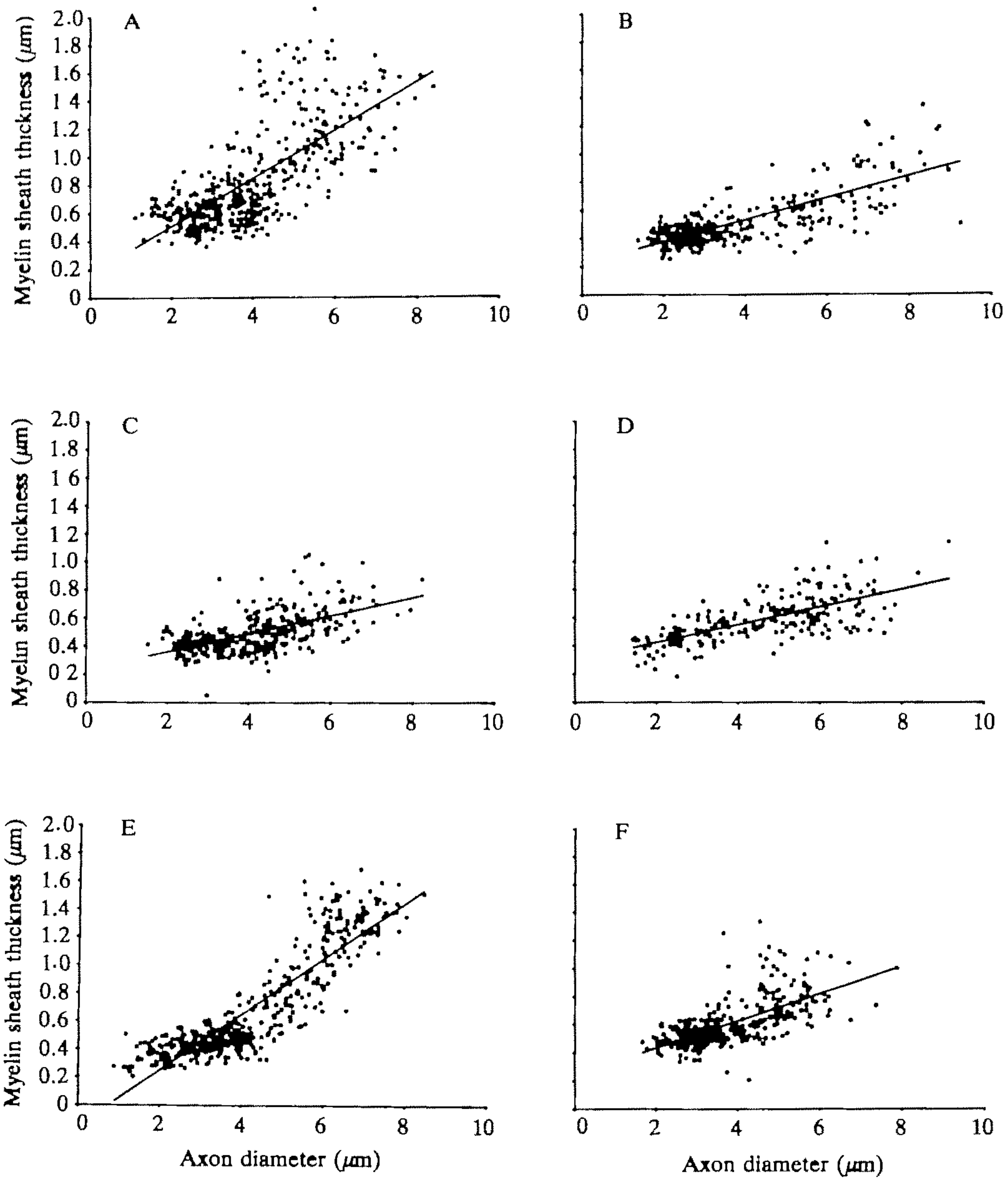

FIG. 4 Scatterplot of myelin sheath thickness against axon diameter A, 5-yr-old control sural nerve, B, *Case 1*, sural nerve; C, *Case 2*, sural nerve; D, *Case 2*, femoral nerve, E, 3-yr-old control sural nerve, F, *Case 3*, sural nerve. The calculated regression lines are shown and the values for these slopes are given in Table 3. Myelin sheaths are thinner in the patients' nerve fibres

Case 1 and in the femoral nerve of Case 2, with maximum fibre diameters similar to those of the control. In the sural nerves of Cases 2 and 3 the distribution was unimodal with the maximum fibre diameters about 1 μm less than their controls. Inspection of

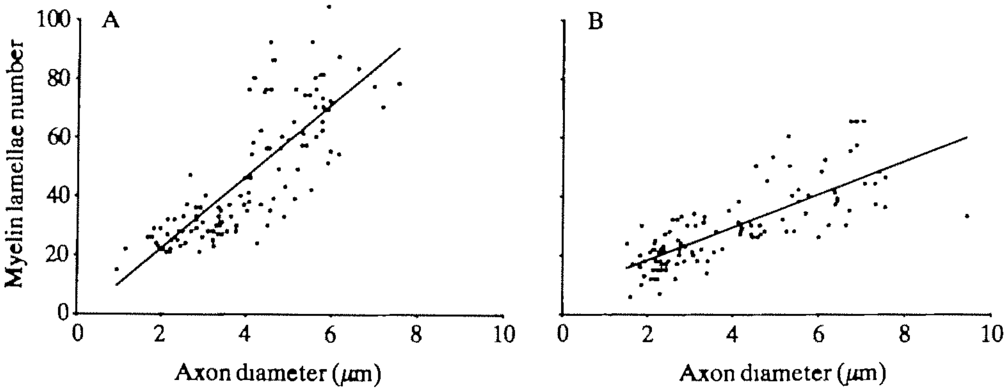

FIG. 5 Scatterplot of numbers of myelin lamellae and axon diameter, with regression lines, from the sural nerve of the 5-yr-old control (A) and Case 1, (B) The slope of B is much flatter than that in the control (A). Values for the slope coefficients are given in Table 4.

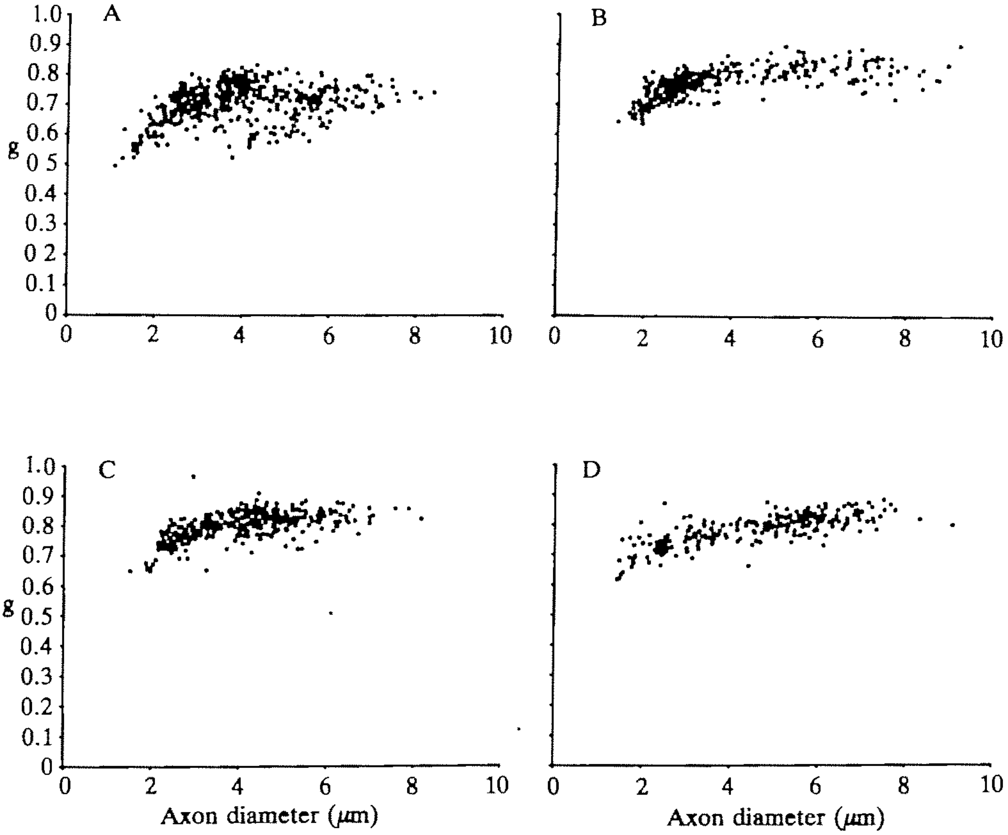

FIG. 6 Scatterplots of g ratio and axon diameters. A, 5-yr-old control; B, *Case 1*, sural nerve, C, *Case 2*, sural nerve, D, *Case 2*, femoral nerve There are more values > 0.8 in B, C and D than in the control (A)

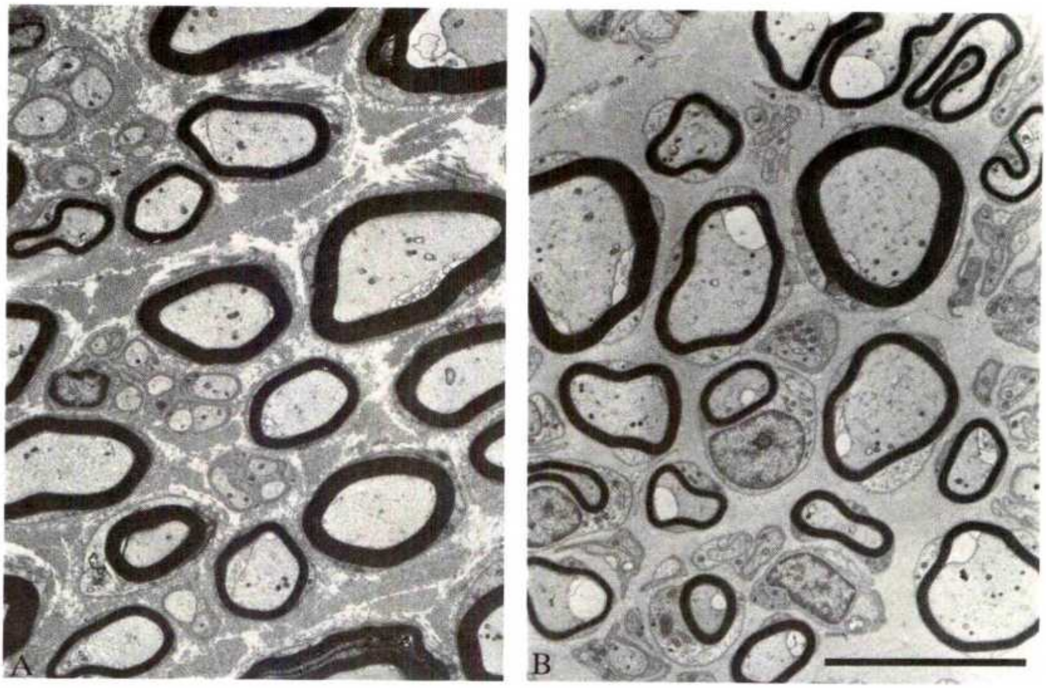

FIG. 7. Electron micrograph of sural nerve from a 5-yr-old control (A) and from Case 1 (B). Several fibres in B are more thinly myelinated than those in A. Bar = 10 μm.

the scatterplots of g ratios and of myelin sheath thickness with axon diameter (figs 4, 5, 6) shows that maximum axon size is similar to that of controls in Cases 1 and 2, but in Case 3 axons are smaller than the control.

Other observations made on tranverse sections included the presence of some fibres of irregular shape and with folded myelin sheaths (*see* fig. 3). In Case 3, fibres were rather smaller than those of the control, with a maximum diameter of 8 μm, as compared with 9 μm for the 3 yr control.

Electron microscopy showed no evidence of fibre degeneration or of active demyelination (fig. 7). There was an increase in endoneurial collagen in each nerve. An occasional myelinated fibre was seen with encircling processes of Schwann cells sometimes associated with unmyelinated axons.

Myelin periodicity of fibres in each nerve was within the normal range (14.50 nm ± 0.60 SD). No abnormal mitochondria were seen in the sural nerve biopsy. No assessment of mitochondrial morphology was made in the nerves taken postmortem.

Unmyelinated axons tended to be in larger groups than normal and their density in all of the nerves was somewhat higher than that of controls (Table 2), suggesting that some regeneration had taken place.

Quantification of teased fibres from control nerves showed a linear relationship between internodal length and fibre diameter, with maximum internodal lengths of 600 – 700 μm.

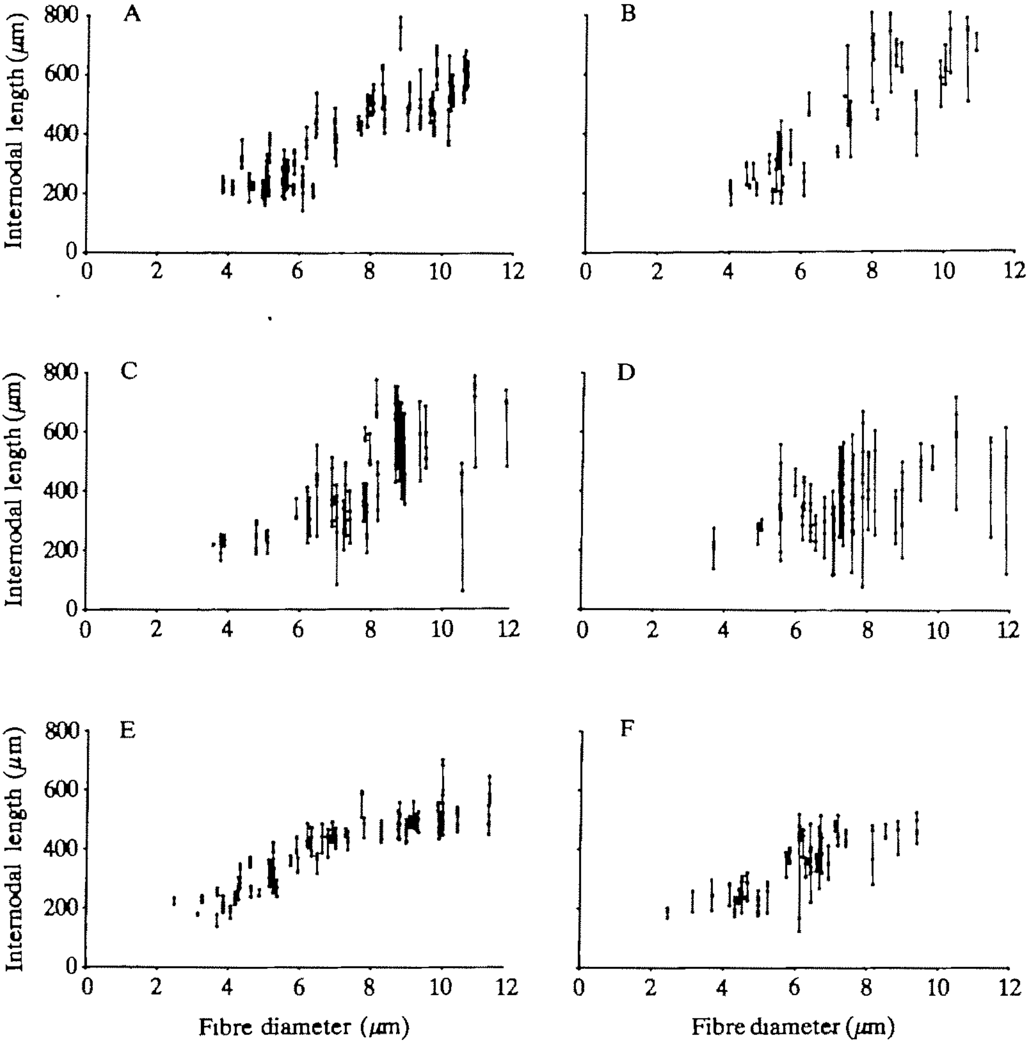

FIG 8 Graphical representation of internodal length and fibre diameter vertical lines join internodal lengths from the same fibre. A, 5-yr-old control sural nerve, B, *Case 1*, sural nerve, C, *Case 2*, sural nerve, D, *Case 2*, femoral nerve, E, 3-yr-old control sural nerve, F, *Case 3*, sural nerve. The normal linear relationship between internodal length and fibre diameter is seen in the controls (A, E), and there is a near normal pattern in B and F. In C there are some fibres with shorter internodes, and these are more numerous in D

This normal linearity was seen in fibres from sural nerves of Cases 1 and 3 (fig. 8B, F) although the range of internodal length for a given fibre size was wider in these nerves than in the respective controls (fig. 8A, E). Maximum internodal length was shorter in Case 3, possibly because the child was of very short stature, and maximum internodal length is known to be closely related to the length of the part in which it lies (Jacobs, 1988).

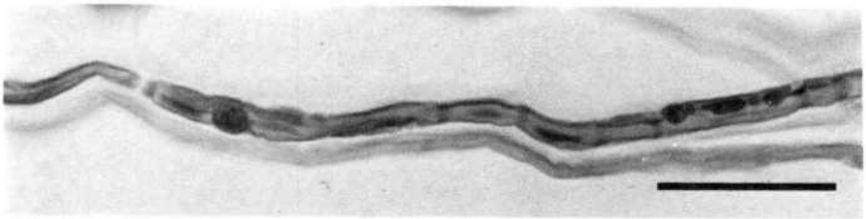

FIG. 9. Teased fibre from sural nerve of *Case 1* showing focal irregularities along an internode which is seen to be only thinly myelinated. Bar = 50 μm.

In the sural nerve of Case 2 there were a few fibres with short internodes, and in the femoral nerve, many more fibres had internodes of variable, short length (fig. 8C, D), suggestive of remyelination. However, 'original' internodes of expected length for fibre diameter could still be identified as having thin myelin sheaths. In all nerves there were some fibres in which myelin sheaths showed focal irregularities and foldings (fig. 9) to a greater degree than seen in control nerves.

DISCUSSION

In 2 of our cases the CNS lesions at autopsy were characteristic of Leigh's disease, and the second CT scan in Case 1 showing bilateral pallidal lesions strongly points to this diagnosis. Histochemical analysis demonstrated deficient activity of cytochrome c oxidase in muscle in Cases 1 and 2 and showed low activity in Case 3. The defect was confirmed in cultured fibroblasts of Case 2. The histochemical and biochemical features, and the clinical similarities of each patient all suggest that we are dealing with a particular subset of the variety of metabolic disorders which may give rise to Leigh's disease. This possibility should not lessen the implication of our findings for the generality of Leigh's cases, since clinical evidence of peripheral neuropathy in Leigh's patients has been reported in association with differing clinical presentations and enzymopathies (Willems *et al.*, 1977; Evans, 1981; Egger *et al.*, 1982; Seitz *et al.*, 1984).

There are few references to nerve conduction changes in the literature of Leigh's disease. The first seems to be that of Robinson *et al.* (1967), who reported a motor nerve conduction velocity (MNCV) of 28 $\mathrm{m \cdot s^{-1}}$ in the common peroneal and ulnar nerves of a 15-month child. Dunn and Dolman (1969) found that a median nerve MNCV of 30 $\mathrm{m \cdot s^{-1}}$ in a 7 yr-old fluctuated with clinical relapses and remissions, and likened it to the abnormality found in infective polyneuritis. Moosa (1975, 1978) reported slowing of ulnar and tibial nerve MNCV in 3 of 4 infants, each studied serially over a year or so. He suggested that slowing might precede clinical expression of the disease and therefore be useful in the diagnosis of asymptomatic siblings. Goebel *et al.* (1986) found slow MNCV in 3 of 4 infants examined. In a review of 173 pathologically proven cases of Leigh's disease (Van Erven *et al.*, 1986) reference was made to EMG findings in 31. Conduction was said to have been slowed in 15, but no differentiation between sensory and motor conduction was mentioned and no figures for velocity given. The authors themselves recorded a further 12 patients, 3 of whom showed electrophysiological evidence of a neuropathy affecting both sensory and motor fibres. No figures were given. They concluded that such neurophysiological studies did not contribute to the diagnosis of Leigh's disease but helped to distinguish it from other diagnostic possibilities.

It seems, therefore, that slowing of conduction of mild to moderate degree is common but not inevitable in this syndrome. The 3 children here reported were investigated before the diagnosis was known. The results were notable for suggesting that demyelination must play a prominent part. In particular it is rare (in our experience) for sensory action potentials to be delayed to the extent found. The F wave in Case 1 was very delayed, and the proximally evoked thenar MAP in Case 2 showed a large decrement. It was therefore of considerable interest, when, after early assessment of sural nerve biopsies (without quantification) had not demonstrated significant involvement of myelin, hypomyelination was eventually demonstrated.

Of the 23 cases of Leigh's disease reviewed by Goebel *et al.* (1986), including 4 of their own, 6 had clinical evidence of peripheral neuropathy but 16 had nerve pathology described as demyelination. Most of the pathology had been assessed from paraffin histology which does not allow critical evaluation of nerve fibre changes. However, Goebel *et al.* examined 4 sural nerve biopsies using semithin resin sections and electron microscopy, with some nerve fibre quantification. By light microscopy, thinness of the myelin sheaths was remarked upon in 3 of the 4 nerves; this was supported quantitatively by the finding of higher g ratios. In none of the biopsies was there evidence of myelin or axonal lesions. Fibre densities were not calculated. By electron microscopy there was some evidence of primary demyelination and remyelination. As pointed out by Goebel *et al.*, data showing high g ratios does not indicate whether the thin myelin sheaths are the result of regeneration or remyelination.

In the present study, there was no evidence of myelinated fibre loss: no degenerating fibres or empty bands of Büngner were seen. Myelinated fibre densities were therefore considered to be within normal limits.

We have shown significantly reduced values for the slope of the regression of myelin sheath thickness (or in 1 case, numbers of myelin lamellae), and axon diameter when compared with age-matched controls. In addition, quantification of teased nerve fibres has shown a normal linear relationship between internodal length and fibre diameter in 2 of the nerves. This would seem to exclude the likelihood of either nerve fibre regeneration or demyelination and remyelination as an explanation for the hypomyelination.

It is known from experimental studies (Vizoso and Young, 1948; Hildebrand *et al.*, 1987) that internodal lengths of fibres regenerating after nerve crush at a very early stage of development eventually display a linear relationship with fibre diameter which closely resembles that of normal fibres. This is in contrast to the uniformly short internodal lengths of fibres regenerating after nerve crush in an adult. The possibility of an early episode of nerve fibre degeneration with subsequent regeneration affecting all fibres is an improbable explanation for the hypomyelinated fibres in Leigh's disease. In view of the virtually normal appearance of teased fibres in two of the nerves, the absence of evidence of demyelination or of degeneration and regeneration, and the normal values found for myelinated fibre density, it seems most likely that nerve fibres in Leigh's disease are more thinly myelinated than normal from the outset.

Focal irregular foldings of the myelin sheaths were seen occasionally in all 4 nerves examined. Possibly these may be associated with some instability of the myelin which eventually results in demyelination, seen in a few fibres in 1 sural nerve, but affecting more fibres in the femoral nerve from the same patient. An analogy could perhaps be made to the more conspicuous focal foldings of myelin sheaths of fibres in tomaculous neuropathy which are associated with segmental demyelination (Behse *et al.*, 1972).

Children with Leigh's disease are usually below normal weight and height, and the question of nerve fibre changes due to failure to thrive should be considered. There are few studies on peripheral nerves of malnourished children but a recent report of protein malnutrition (Chopra *et al.*, 1986) showed that in mild to moderate cases (with body weight 61–80% of expected weight), there was normal development of myelinated fibres and an appropriate relationship of internodal length to fibre diameter. Myelin sheath thickness was not measured. Children with severe protein-calorie malnutrition showed abnormalities of the normal developmental pattern for myelinated fibre distribution. Although there was a reduction in motor nerve conduction velocity and abnormalities of sensory conduction velocity in both moderately and severely affected children, the changes were generally not significantly different from controls. Neither morphological nor electrophysiological abnormalities suggest that malnutrition causes generalized hypomyelination.

Clinical symptoms of peripheral neuropathy are only occasionally reported in Leigh's disease, but the incidence of electrophysiological abnormality is considerably higher (*see* Goebel *et al.*, 1986), and this, possibly in combination with nerve biopsy, may be a useful aid to the diagnosis of Leigh's disease. The reduced conduction velocities recorded in peripheral nerves in Leigh's disease may be due both to hypomyelination of the myelin sheaths and to segmental demyelination.

Finally, it is of interest that in other conditions in which there is mitochondrial cytopathy, included under the heterogeneous group known as chronic progressive external ophthalmoplegia, there are some reports of slowing of conduction in peripheral nerves (Drachman, 1968; Gemignani *et al.*, 1982; Cantello *et al.*, 1985). Peyronnard *et al.* (1980) reported a case of 'ophthalmoplegia-plus' syndrome, with severe sensorimotor neuropathy and reduced conduction velocities. A nerve biopsy showed loss of axons, but the remaining fibres were found to have disproportionately thin myelin sheaths (confirmed quantitatively), without evidence of demyelination.

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ADDENDUM

Case 1 has since died at the age of 11 yrs 9 mos. Neuropathological examination shows CNS lesions similar to those found in her sister, Case 2.

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DISORDERS OF EYE MOVEMENT IN MYOTONIC DYSTROPHY

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SUMMARY

Horizontal saccades and smooth pursuit eye movements were studied in 26 patients with myotonic dystrophy. Clinical neuro-ophthalmological investigations in 1 patient revealed an inability to achieve a full range of eye movements Electro-oculography showed a significant decrease of the maximum velocity of the visually-guided saccades in 83% of the patients. Smooth pursuit eye movements were not significantly different from age-matched controls. Visual evoked potential (VEP) latencies (P100) were significantly prolonged compared with controls in 64% of the patients. The saccadic latency of the visually-guided saccades was correlated with the prolonged VEP latencies, indicating that lesions in the primary visual pathways probably contribute to the oculomotor dysfunction. The isolated decrease of the maximum velocity of the saccades in combination with EMG findings favours a peripheral (dystrophic) pathophysiological mechanism.

INTRODUCTION

Myotonic dystrophy (MD) is an autosomal dominant multisystem disorder with variable penetrance, in which dystrophic changes, apart from the predominant muscle involvement, are also evident in the central and peripheral nervous system (Jamal *et al.*, 1986). Patients with MD seldom complain of diplopia and clinical neuro-ophthalmological investigation rarely reveals any disturbance of eye movement except for some ptosis. Electro-oculographic (EOG) studies on disorders of eye movement in MD have so far yielded conflicting interpretations as to the underlying pathophysiological mechanisms. Decreased maximum velocity of small and large amplitude saccades has been reported in limited series of patients (Baloh *et al.*, 1975; Oohira *et al.*, 1985), sometimes more pronounced for horizontal than vertical eye movements (Emre and Henn, 1985). Other oculomotor changes include hypometric saccades and disturbances of smooth pursuit (von Noorden *et al.*, 1964; Burian and Burns, 1967; Lessell *et al.*, 1971). Some authors have suggested a central origin for the eye movement disorders (von Noorden *et al.*, 1964; Burian and Burns, 1967; Lessell *et al.*, 1971; Emre and Henn, 1985) while others have considered that they are related to a more peripheral mechanism (Junge, 1966; Oohira *et al.*, 1985).

We have investigated 26 MD patients in whom we undertook routine neuro-ophthalmological investigation, EOG, visual evoked potential (VEP) and electromyographic (EMG) studies on the extraorbital muscles. VEP and EMG of the extraorbital muscles

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were examined to establish to what extent an afferent visual deficit and a myopathic disorder of the external ocular muscles, respectively, might be involved. One study (Gott *et al.*, 1983) reported bilateral prechiasmatic VEP abnormalities in 17 MD patients; no precise localization of the lesion was given. EMG of the external ocular muscles has revealed myopathic changes similar to those in the limb muscles, sometimes accompanied by myotonic discharges (Davidson, 1961; Zauberman and Magora, 1969). The primary objective of our study was to ascertain whether a characteristic pattern of subclinical oculomotor disorder could be detected in patients with MD. Special attention was paid to the question of central versus peripheral pathophysiological mechanisms.

PATIENTS AND CONTROLS

Patients

Twenty-six patients with MD, 15 males and 11 females, were studied, with an age range of 11–70 (mean 38) yrs. The duration of the clinical symptoms varied from 4 months to 36 yrs. The diagnosis of MD was made from the clinical features, confirmed in all cases by EMG. A positive family history was present in all. None of the patients took any drugs which could influence myotonia, the EOG or VEPs.

Controls

Normal values were obtained from healthy control subjects recruited from the staff of the St Elisabeth Hospital and their relatives. There were 25 control subjects for the VEP studies, matched for age and sex, and 20 for EOG also matched for age and sex.

METHODS

Routine neurological examination

This was performed with special attention to eye movements (saccades, on command and visually-guided, smooth pursuit, myotonia), ptosis and specific MD symptoms (muscle weakness, atrophy, myotonia).

Ophthalmological investigations

These included the measurement of visual acuity by an Oculus-Visutest apparatus which was expressed in decimal values, funduscopy, extraocular movements and a slit lamp examination for lens opacities.

Electro-oculography

Eye movements were measured by d.c. electro-oculography (EOG). Nonpolarizable miniature silver-silver chloride skin electrodes were placed on the inner and outer canthi of the eyes to permit separate recordings for each eye. The signals from the electrodes were displayed on an ink-jet writing polygraph and analysed quantitatively with a PDP 11 computer. Patients were seated in a darkened room and their heads were stabilized by an occipital support.

Horizontal visually-guided saccades and horizontal smooth pursuit movements were studied. Targets for saccades consisted of red light-emitting diodes fixed on a cylindrical screen. The positions were 10°, 20° and 30° to the left and right of the primary position. Two different testing paradigms for saccades were used: (1) random stimulation with targets of variable amplitude; (2) stimulation with only the 20° targets to the left and right in a predicted sequence. For the random stimulation programme, only the 20° steps were analysed. The rate of target alternation in the predictive sequence had a pause of 3 s. A filter of 0–70 Hz and a sampling frequency of 512 Hz were used for the recording system. A complete saccade test consisted of at least 20 steps of 20° to both sides. The parameters studied included saccadic latency, maximum saccadic velocity (V_{max}) and amplitude.

Smooth pursuit movements were elicited by a target moving on a dark background. The target was guided by a laser beam reflected by a mirror galvanometer. The target moved in a triangular wave fashion with excursions of 50° (25° left and right out of the midposition). The velocities varied from 30 to 50 deg/s. The filter range was 0–20 Hz. Smooth pursuit was quantitated by measuring the pursuit gain (the ratio of pursuit eye velocity/target velocity) Smooth pursuit gain was obtained by calculating the gain, along a trajectory ranging from −15° to +15° from the midposition, between sample points with an interval of 0.02 s. The average gain of the velocities of 30–40–50 deg/s was determined.

VEP recording. VEPs were measured by the checkerboard method (Halliday, 1982) with 129 image reversals at a rate of 1 Hz, twice to the left and twice to the right The checkerboard measured 27×31 cm. Each square subtended 48 min; the full field pattern was 10° 25′ Full-field luminance was 40 cd/m^2 with a background luminance of 0.05 cd/m^2. The recordings were performed with the subject seated in a dimmed room, 80 cm from the screen. Each eye was tested twice to ensure replicability of the wave form. The latencies were determined for the major occipital positive peak (P100).

Electromyography. Needle EMG of levator palpebrae and the external recti was performed unilaterally with special attention to insertion and spontaneous activity, the amplitude of motor unit potentials, myotonic discharges and the voluntary recruitment pattern.

RESULTS

Neurological examination and ophthalmological investigation were undertaken in all cases. VEPs were recorded in 26 patients (100%), EOG in 18 (69%) and EMG in 17 patients (65%). The disorder varied in severity, being mild in 24%, moderate in 16%, moderate/severe in 28% and severe in 32% (Rankin disability scale). Clinically evident myotonia was demonstrable in the limb muscles in all cases. One patient showed an external ophthalmoplegia with limitation of external ocular movements. Bilateral ptosis in some degree was present in 17 cases (65%). Ophthalmological examination demonstrated impaired visual acuity in 18 patients (69%). Two patients had unilateral amblyopia, accompanied by a strabismus of less than 5°; 5 patients had decreased vision below 0.3, 1 unilaterally and 4 bilaterally. Cataract was found in 17 patients (65%) and typical myotonic butterfly-shaped macular dystrophy in 1 case. The reduced visual acuity was not always related to the cataract as this sometimes persisted after successful cataract excision. Ocular hypotonia was not found, nor was disc pallor detected.

The results of the neurophysiological investigations are summarized in Tables 1–3. Values in excess of 2 SD were considered as abnormal.

The P100 VEP latency was significantly prolonged in 64%, without obvious left-right differences, indicating a bilateral prechiasmatic abnormality. If corrected for a visual acuity above 0.3 the P100 latency was abnormal in 44%. The P100 latency did not correlate with age or presence of cataract; after cataract surgery prolonged P100 latencies were also noted.

The EOG was significantly abnormal in 83% of the MD patients. The most striking disorder was a significant decrease of the maximum velocity of the visually-guided saccades (83%). The latencies of the saccades were slightly increased but compared with the controls this difference was not significant (figs 1, 2). This prolongation was not significantly different between the first (mean latency: 242 ms, SD 65.8), fifth (mean latency: 253.6 ms, SD 74.9) and tenth (mean latency: 277.3 ms, SD 62.7) saccade in the predicted sequence trial. No significant difference of latency was observed in the random trial following saccades of different amplitudes: mean saccadic latency, following a 10° saccade, was 267.8 ms (SD 65.1) and following a 30° saccade 267.1 ms (SD

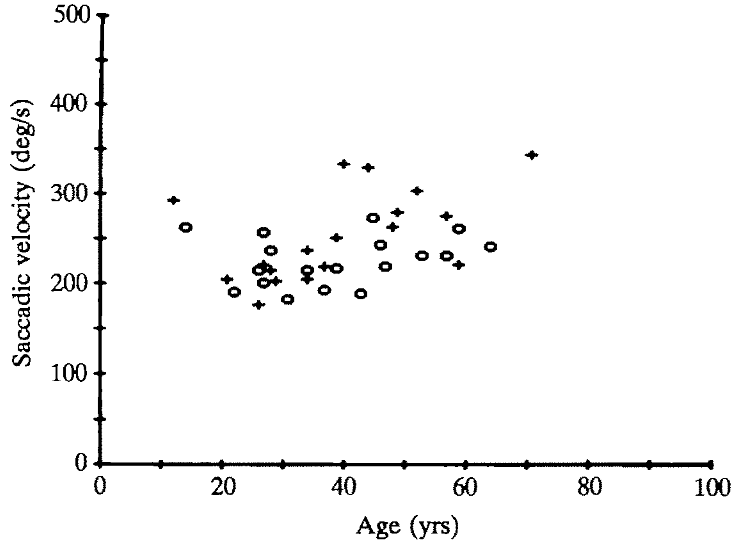

FIG 1 Latency of visually-guided saccades of patients with MD and age-matched controls showing slightly prolonged latencies in the patient group although not significantly different from the controls. Patients (crosses), controls (open circles).

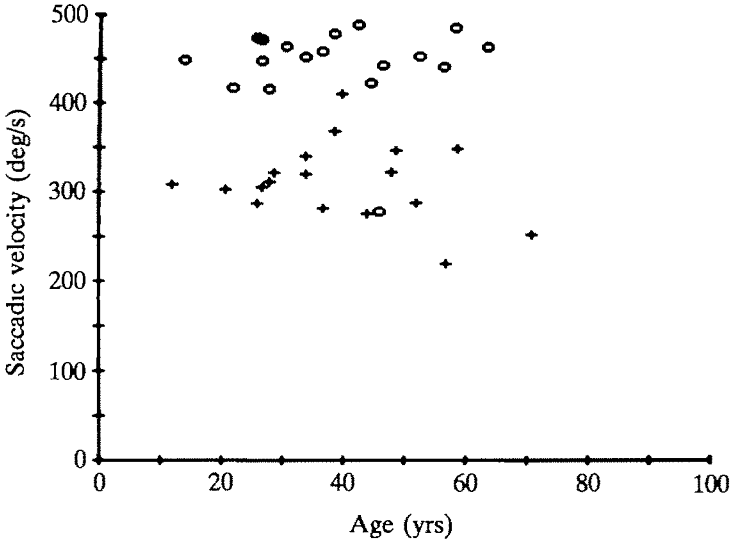

FIG 2 Decreased V_{max} of visually-guided saccades of patients wtih MD as compared with age-matched controls. Note the highly significant slowing of the saccade velocity in MD patients ($P < 0.006$). Patients (crosses), controls (open circles).

63.9). The amplitudes of the saccades were within normal limits (Table 1). The latency of the P100 correlated significantly with the latency of the visually-guided saccades in the MD group ($P = 0.01$; R value, 0.455) (fig. 3), while in the control group no significant correlation could be detected. Linear regression analysis of EOG latency/VEP latency between patients and presumed normals showed no significant difference ($P = 0.09$). The V_{max} of the visually-guided saccades was not influenced by age, either for presumed normals or for MD patients. The most striking finding was the significant ($P < 0.006$) difference in V_{max} of visually-guided saccades between presumed normals (mean: 442.7 deg/s, SD 46.7) and MD patients (mean: 309.7 deg/s, SD 43.2) (*see* fig. 2). This was independent of the testing paradigm. A substantial variation in the amplitude of the saccades could not account for the decrease of V_{max}. No central eye movement disorders such as dysmetria, nystagmus or gaze palsy were encountered. The smooth pursuit gain (the ratio of pursuit eye velocity/target velocity) showed no significant abnormalities (*see* Table 1).

The results of EMG of the extraocular muscles are listed in Table 3. No neurogenic

TABLE 1 EOG FINDINGS IN PATIENTS WITH MYOTONIC DYSTROPHY

	Controls		*Patients*		
	Mean	*SD*	*Mean*	*SD*	*Wilcoxon test*
Saccades					
Latency	225.1 ms	27.7	252.7	50.4	$P < 0.1074$
V_{max}	442.7 deg/s	46.7	309.7	43.2	$P < 0.006$(S)
Mean amplitude gain					
R eye	0.90	0.07	0.91	0.07	
L eye	0.85	0.07	0.90	0.08	
Smooth pursuit					
Mean gain (30–50 deg/s)	0.82	0.14	0.72	0.22	$P < 0.131$

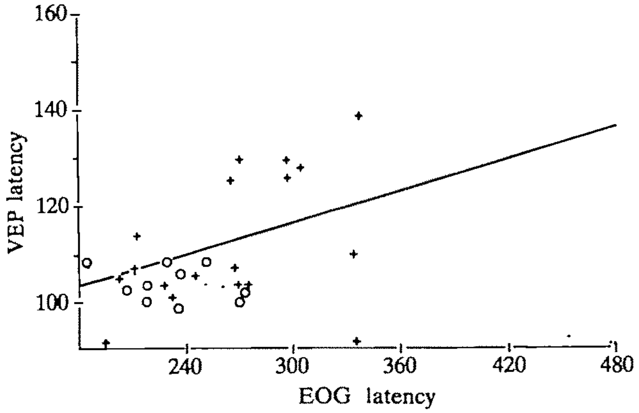

FIG 3. Significant correlation between P100 latency of the VEP and the latency of the visually-guided saccades ($P = 0.01$) in the MD patient group but not in healthy controls ($P = 0.14$). No statistically significant difference is detectable between the two groups Patients (crosses), controls (open circles)

abnormalities were found and the recruitment pattern on maximum effort was within the normal range. The duration of the action potentials was rather short with a relatively low voltage. Myotonic discharges were regularly observed.

DISCUSSION

Our results indicate the presence of a significant subclinical disorder of eye movement in patients with MD (Table 1). The most striking finding consists of a decrease of V_{max} of the horizontal visually-guided saccades compared with normal subjects ($P < 0.006$, Wilcoxon test), which does not correlate with age (fig. 4). This finding is in agreement with reports in the literature (Baloh *et al.*, 1975; Oohira *et al.*, 1985). However, all

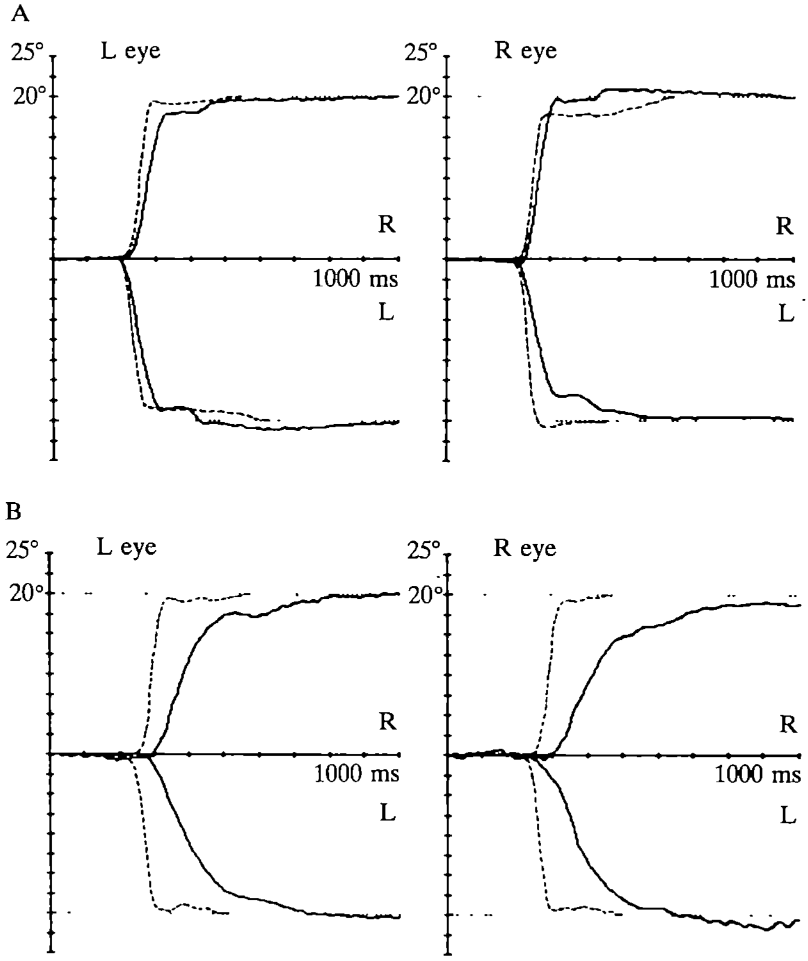

FIG. 4. Examples of recordings of visually-guided saccades of slightly affected (A) and severely affected (B) MD patients, both compared with an age and sex-matched control subject (dotted line). Note the slow saccadic velocity.

other parameters for visually-guided saccades and the smooth pursuit movements are within normal limits, which is in contrast with most earlier reports in the literature (von Noorden *et al.*, 1964; Burian and Burns, 1967; Lessell *et al.*, 1971).

The latency of the visually-guided saccades is slightly increased, although compared with controls the difference is not statistically significant. This latency correlates with the latency of the P100 of the VEP ($P = 0.01$; r value, 0.455; fig. 3) only in the patient group, not in the controls. However, no statistically significant difference could be detected between the groups, possibly due to the limited number of patients and based on a type II error. The prolonged latency of the saccades thus possibly reflects an afferent visual disturbance as an explanation. The bilateral prolongation of the P100 latency of VEP ($P < 0.001$, Wilcoxon test), suggests a bilateral prechiasmatic deficit (Table 2), the localization of which is uncertain (Gott *et al.*, 1983).

TABLE 2. VEP IN MYOTONIC DYSTROPHY

Myotonic dystrophy Age 11–77 yrs	Mean (R/L) 111/115 ms	SD 12/17 ms
Controls Age 18–60 yrs	Mean (R/L) 100.8/100.9 ms	SD 4.5/3.6 ms

Smooth pursuit eye movements are not significantly different from controls, as evidenced by the normal gain. In a number of cases a few catch-up saccades were seen. This phenomenon often indicates a reduced gain, but the smooth pursuit gain in our patient group was not significantly different from age-matched controls. Apparently our findings confirm the presumption of Oohira *et al.* (1985) that MD patients have a smooth pursuit gain that is within normal limits, although frequently interrupted by corrective saccades. This does not, however, always imply a CNS disorder (Schalén, 1980; Schalén *et al.*, 1982; Leigh and Zee, 1983).

Abnormal smooth pursuit findings were the reason for previous authors considering the eye movement disturbances as being of central origin (von Noorden *et al.*, 1964; Burian and Burns, 1967; Lessell *et al.*, 1971). Disorders at different levels of the peripheral and central nervous systems have been described in MD patients (Refsum *et al.*, 1967; Lundervold *et al.*, 1969; McComas *et al.*, 1971; Ballantyne and Hansen, 1975; Panayiotopoulos and Scarpalezos, 1977; Jamal *et al.*, 1986), involving, for instance, the brainstem. This has been demonstrated by evoked potential studies (Thompson *et al.*, 1983; Bartel *et al.*, 1984). The slow saccades, as seen in our patients, may be due to a disorder of the saccadic burst cells in the pontine reticular formation (Leigh and Zee, 1983). However, the absence of abnormalities of eye movement with respect to saccadic latency and amplitude and normal smooth pursuit gain makes this explanation unlikely (Leigh and Zee, 1983). The same is true for a supranuclear deficit because this also leads principally to abnormalities of the latency and amplitude of saccades (Schiller *et al.*, 1980). An ocular motor nerve paresis is characterized by slow hypometric saccades. The saccades become slower as they move in the field of action of the paretic muscle. This has not been found in our patients. The isolated finding of a decreased saccadic velocity in patients with MD is therefore in favour of a peripheral myopathic pathophysiological mechanism. The EMG results (*see* Table 3) for the extraocular muscles demonstrating myopathy and myotonic discharges in a number of patients support this conclusion. The eye movement disturbances indicate a 'dystrophic' rather than a myotonic abnormality. In the predictive sequence of the saccades there was no change during the block of trials and there was no significant difference of saccadic latency in the at random trial following saccades of different amplitudes.

TABLE 3 EMG OF THE EXTRAOCULAR MUSCLES IN MYOTONIC DYSTROPHY

Insertion activity	Myotonic discharges 35%
Action potential characteristics	LP amplitude· mean 560 μV (N = 400–1200 μV) RE amplitude. mean 520 μV (N = 300–1000 μV) LP duration: mean 1 8 ms (N = 1–3 ms) RE duration. mean 1.8 ms (N = 1–3 ms)
Myotonic discharges	LP· 0%, RE 35%

LP = levator palpebrae RE = external rectus N = normal values.

The organization and structure of the extraocular muscles is quite different from that of other skeletal muscles in man (Miller, 1985). Different types of fibres are identifiable in human extraocular muscles by a variety of means, including light and electron microscopy (Kato, 1938; Dietert, 1965; Brandt and Leeson, 1966; Peachey, 1971; Bastiaensen, 1978), histochemistry (Ringel *et al.*, 1978; Bormioli *et al.*, 1979; Hoogenraad *et al.*, 1979) and by pharmacological and electrophysiological properties (Scott and Collins, 1973).

According to Ringel *et al.* (1978), fibre types have been labelled based on histochemical features, as 'coarse', 'fine' and 'granular'. Coarse fibres, with a high myosin ATPase activity and high oxidative enzyme content, are predominant in the periphery of the extraocular muscles. Under the electron microscope these fibres show a 'Felder Struktur', with large fields of myofibrils. The coarse fibres are supplied by many small calibre (gamma) nerve fibres with multiple 'en grappe' nerve endings (Bastiaensen, 1978; Ringel *et al.*, 1978). The fibres with a fine granular appearance (fine fibres) are more regularly distributed throughout the muscle and contain a moderate amount of myosin ATPase and oxidative enzymes. Granular fibres predominate in the central parts of extraocular muscles. They show a high myosin ATPase activity and a low oxidative enzyme content. On electron microscopy, these fibres show a 'Fibrillen-Struktur' with grouped myofibrils. The granular and fine fibres are singly innervated by large motor neurons with 'en plaque' nerve endings (Bastiaensen, 1978; Ringel *et al.*, 1978).

The physiological action of the different extraocular muscle fibres correlates with the type of nerve endings seen by electron microscopy. The large (granular and fine) muscle fibres with en plaque nerve endings show fast (twitch) activity as required for saccades. The small (coarse) muscle fibres with en grappe nerve endings show a more tonic activity as required for smooth pursuit and the slow phase of nystagmus (Kato, 1938; Hess and Pilar, 1963; Bastiaensen, 1978; Ringel *et al.*, 1978). The isolated decrease of V_{max} suggests a selective abnormality of the fibres with en plaque nerve endings.

The decrease of V_{max} with normal saccade amplitudes is in agreement with the results of Baloh *et al.* (1975) who tested 3 patients with MD and stated that in the mildest form of this disease, without any visual complaint, the ocular motor involvement consists of slowed saccades in patients who have a full range of eye movements. Exceptionally, external ophthalmoplegia or gaze palsy is caused by myotonic dystrophy (Lessell *et al.*, 1971; Miller, 1985). One patient in this study clinically had an external ophthalmoplegia with markedly slowed horizontal saccades and a limited range of eye movements in all directions. Generally, the preservation of a full range of eye movements separates the MD group from the ocular myopathies.

Finally, there is a deficit of the afferent visual system, as shown by the VEP, in a large percentage of the patients, the lesion probably being located prechiasmatically. The exact localization of this disorder is the topic of a current study.

Conclusions

The results of application of EOG, VEP and EMG studies to the extraocular muscles show clearly that subclinical disorders of eye movement are frequently present in MD patients. These consist of an isolated decrease of V_{max} of the visually-guided saccades. As other parameters of saccades and smooth pursuit are within normal limits, this indicates a disorder of myopathic origin.

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DIRECTIONAL HYPOKINESIA AND HEMISPATIAL INATTENTION IN NEGLECT

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SUMMARY

Subjects with neglect of left hemispace may err to the right when bisecting lines. This error has been attributed either to a directional hypokinesia (failure to execute movements fully in or towards the contralateral hemispace) or to a failure to attend to or represent sensory information. Four subjects with neglect were tested on a line bisection task in which these two hypotheses were differentiated by preventing direct viewing of the line and instead using a video camera and monitor, each of which could be moved independently into right or left hemispace. The performance of 2 subjects was consistent with the predictions of the directional hypokinesia hypothesis; the performance of the other 2 subjects was consistent with the attention-representation deficit hypothesis. These data suggest that both a directional hypokinesia and an attention-representation deficit may each be a primary determinant of neglect.

INTRODUCTION

Subjects with neglect of the left side may err to the right when bisecting lines for at least two reasons. First, even in the absence of hemianopia, these subjects may fail to use fully sensory information presented on the left. Secondly, they may fail to initiate, or execute fully, movements into or towards neglected hemispace. The failure to use sensory information from the left may be related either to an attentional or a representational deficit. According to at least one version of the representational deficit hypothesis, neglect of the left side might be attributed to an inability to generate an internal map or sensory representation of a percept (Bisiach and Luzzatti, 1978; Bisiach *et al.*, 1979). Based on this hypothesis, subjects may be assumed to bisect accurately the portion of the line they perceive and represent and neglect the portion of the line that is not represented. According to the representational hypothesis, neglect would be induced by injury to structures that generate and maintain the abstract internal representation of the visual world. Support for the representational theory comes from studies demonstrating that subjects with neglect have retrograde (Bisiach and Luzzatti, 1978) and anterograde (Bisiach *et al.*, 1979; Heilman *et al.*, 1974) memory defects.

Neglect may also be associated with a disorder of directed attention (Posner *et al.*, 1987; Coslett *et al.*, 1988). According to this hypothesis, failure on a line bisection task, for example, may be related to an impairment in *attending* to sensory information

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presented in left hemispace (Heilman and Valenstein, 1979). Although the neuronal apparatus for storing the representations is intact, information is not fully processed and, therefore, not represented. Alternatively, the stimulus may be adequately represented but subjects may be impaired in allocating attention to the left side of the internal representation (Meador *et al.*, 1986). The theories briefly described above may collectively be designated the 'attention-representation deficit hypothesis'.

An alternative explanation for impaired performance on a line bisection task is that subjects are impaired in the initiation or execution of *movements* into or towards the neglected hemispace. Watson *et al.* (1978) first demonstrated that hypokinesia contributes to neglect. After training monkeys to respond to a stimulus by moving the *contralateral* arm, these investigators induced neglect by means of a frontal arcuate lesion. When stimulated on the leg contralateral to the lesion—that is, the 'neglected' leg—animals with neglect were noted to respond normally; when stimulated on the ipsilateral leg, however, the monkeys failed to respond with the contralateral arm. Similar results were obtained in monkeys in which neglect was induced by parietotemporal lesions (Valenstein *et al.*, 1982). More recently, Watson *et al.* (1986) have demonstrated normal sensory thresholds on the neglected extremity in monkeys with parietotemporal lesions. These results cannot be explained by theories that attribute neglect to an impairment in sensory processing and were interpreted as evidence of a defect in intention or the process by which the animal prepares for action.

Although not conclusive, data from brain-injured subjects were also consistent with the directional hypokinesia hypothesis. Heilman *et al.* (1985) demonstrated that subjects with neglect of left hemispace were significantly slower than normal controls or left hemisphere-injured subjects without neglect in the initiation and completion of movements towards the contralateral hemispace. Similarly, Heilman *et al.* (1980) and Girotti *et al.* (1983) have demonstrated that subjects with neglect generate hypometric saccades when attempting to direct their eyes into the neglected hemispace.

Thus even though there is evidence supporting both the hemisensory attention-representation deficit and directional hypokinesia hypotheses, the relative contribution of these factors to the phenomena of neglect in humans remains unclear. One reason for this uncertainty is that the effects of the hemispace of motor response and hemispace of sensory input are typically confounded. Consider, for example, performance on a line bisection task. Stimuli presented in left hemispace are not only bisected in left hemispace but visual information guiding this action is provided in left hemispace. In this condition, the hemisensory attention-representation deficit and directional hypokinesia hypotheses generate the same prediction: subjects are expected to err to the right when stimuli are presented and bisected in left hemispace.

In the present investigation, however, the potential effects of the hemispaces of motor activity and sensory feedback were dissociated by preventing direct visualization of the stimulus and using a video camera and monitor to provide visual feedback. As the monitor could be placed in either right or left hemispace and the motor activity could be performed in either hemispace, subjects could bisect lines in right or left hemispace but receive visual feedback from the same or opposite hemispace. Thus effects attributable to the hemispace of visual input could be dissociated from those attributable to the hemispace in which the patient acted.

Using this paradigm, the predictions generated by the hemisensory attention-representation deficit and directional hypokinesia hypotheses are readily distinguished. Specifically, the former hypothesis predicts a greater impairment with visual feedback from the left hemispace regardless of the hemispace in which the line is bisected; the latter hypothesis, in contrast, predicts worse performance when lines are bisected in left hemispace, regardless of the hemispace of visual feedback. The present investigation was undertaken to test the directional hypokinesia and hemisensory attention-presentation hypotheses.

SUBJECTS

The subjects were selected on the basis of having left-sided neglect on a line bisection task and the ability to cooperate in an extensive testing protocol. We found 4 subjects who met these criteria.

Case 1

A 72-yr-old former accountant suddenly developed weakness and sensory loss on the left side. Initial neurological examination revealed him to be lethargic, with a dense left hemiparesis and profound trimodal neglect. When reexamined approximately 30 wks later, he was alert and orientated with normal speech. His visual fields were full to confrontation and there was no extinction of visual stimuli on the left. He had a left hemiplegia; no elementary sensory deficit was noted but he extinguished auditory and tactile stimuli on the left. His drawings and performance on a letter cancellation task demonstrated neglect of left hemispace. Finally, he demonstrated neglect on a line bisection task; he erred to the right by an average of 9.7 mm when bisecting 32 lines varying in length from 5 to 20 cm placed directly in front of him. Performance was influenced by the hemispace in which the line was presented; he erred to the right an average of 19.2 mm when bisecting 32 lines in left but only 4.8 mm when bisecting 32 lines in right hemispace. The clinical diagnosis was ischaemic infarction in the right middle cerebral artery distribution. The testing reported here was performed approximately 7 months after his infarct. A CT scan with contrast performed 10 days after the onset of symptoms demonstrated an enhancing right frontoparietal infarct with subcortical extension to involve the putamen (fig. 1A, B).

Case 2

A 60-yr-old man noted the sudden onset of left-sided weakness and lethargy. The initial neurological examination revealed right gaze preference, normal visual fields, a mild left hemiparesis and a failure to respond to tactile stimuli on the left. A left-sided reflex preponderance and a left extensor plantar response were noted. His drawings revealed evidence of left-sided neglect; he erred to the right an average of 6 mm when bisecting 16 lines varying in length from 8 to 32 cm in the midline; when bisecting 16 lines in left hemispace he erred to the right an average of 10 mm but erred only an average of 3 mm when bisecting 16 lines in right hemispace. The clinical diagnosis was ischaemic infarction involving the right frontal and parietal lobes. The testing reported here was performed 1 wk after the infarct. A CT scan performed 2 wks after admission revealed a frontotemporal infarct on the right (fig. 2A, B).

Case 3

A 57-yr-old woman with a history of diabetes and hypertension noted left-sided weakness and sensory loss on waking. Neurological examination on admission revealed the patient to be alert with a right gaze preference and a failure to orient to the left. She manifested a moderately severe spastic hemiparesis on

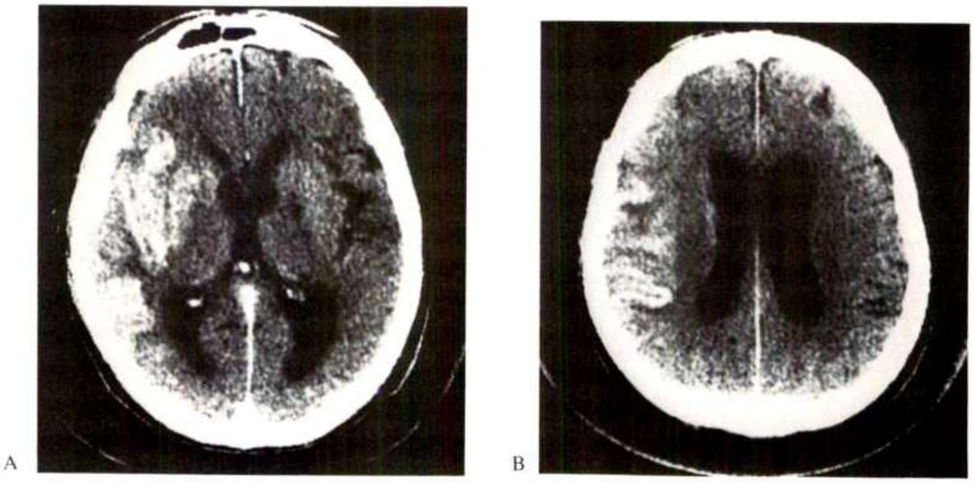


FIG. 1. A, CT scan with contrast from *Case 1* demonstrating infarction of the precentral, superior and middle temporal gyri with involvement of subcortical structures including the putamen on the right. B, higher section from *Case 1* demonstrating infarction of the precentral and postcentral gyri as well as portions of the supramarginal and superior temporal gyri.

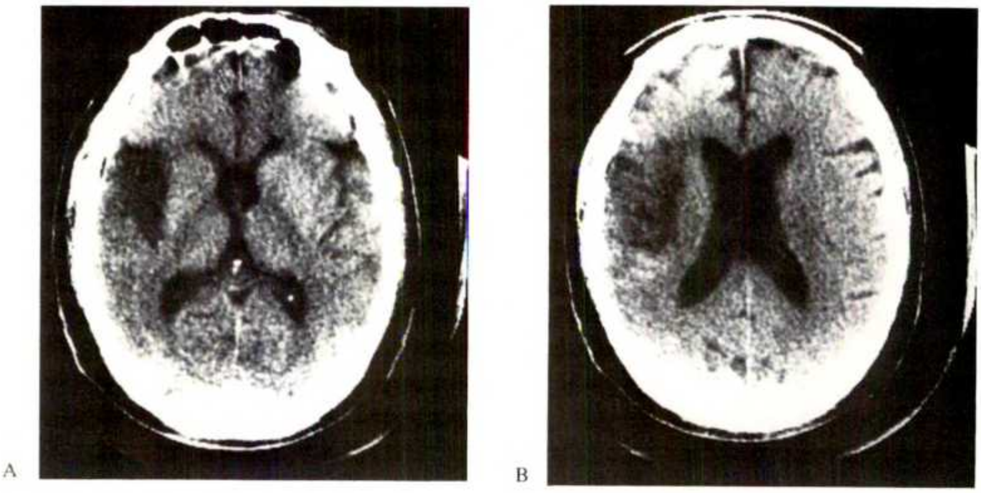


FIG. 2. A, unenhanced CT scan from *Case 2* demonstrating infarction involving the right superior temporal and precentral gyri. B, higher section from *Case 2* demonstrating infarction of the inferior frontal, precentral, postcentral and possibly part of the supramarginal gyri.

the left as well as visual extinction and impaired sensation to all modalities on the left. She omitted the left side in her drawings and erred to the right by an average of 15 mm when bisecting 16 lines presented directly before her. Like Cases 1 and 2, performance on the line bisection task was worse when bisecting lines in left (mean error of 24.2 mm) as compared with right (mean error of 4.7 mm) hemispace. The clinical diagnosis was ischaemic infarction of the right hemisphere. The testing reported here was performed in two sessions approximately 2 wks after the infarction; at the time of testing, the visual fields were full to confrontation but she occasionally failed to report a stimulus presented on the left with double simultaneous stimulation. A CT scan performed 1 yr after the onset of symptoms revealed an infarct involving the posterior temporal and lateral temporooccipital areas as well as small portions of the posterior inferior parietal lobule (fig. 3A, B).

Case 4

A 70-yr-old man with a 7 yr history of focal motor seizures involving the right arm noted the sudden onset of left-sided weakness and sensory loss. Physical examination at the time of admission revealed the patient to be lethargic but orientated; the visual fields were full to confrontation but he exhibited extinction on the left. Pyramidal clumsiness of the right hand as well as clumsiness and mild weakness of the left arm and leg were also noted. Light touch, pain, graphaesthesia and two-point discrimination were diminished on the left; extinction of suprathreshold tactile stimuli was also noted on the left. The tendon reflexes were abnormally brisk in both arms and the left leg. The patient's drawings exhibited hemispatial neglect of the left side; he erred an average of 18.2 mm to the right when bisecting 32 lines in the midline and 33.4 and 7.3 mm when bisecting 32 lines in left and right hemispace, respectively. The testing reported here was performed from 1 to 3 months after the patient's right hemisphere infarct.

A CT scan performed at the time of admission revealed small cortical infarcts involving the precentral and, perhaps, postcentral gyri on a supraventricular section and superior portions of the occipital lobe on the left. A CT scan performed 3 months later demonstrated the old left hemisphere infarcts as well as an extensive infarct involving the right temporal lobe and extending into the parietal and posterior frontal lobes on the right (fig. 4A, B).

Control subjects

Five subjects without clinical neglect served as controls. Case C1 was a 63-yr-old male with a dense left homonymous hemianopia and left-sided sensory impairment secondary to an extensive right occipital

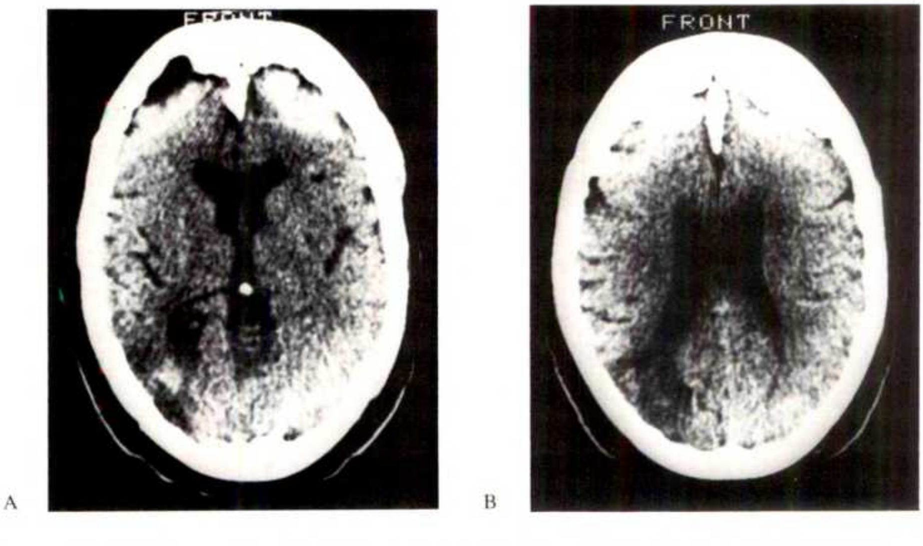


FIG. 3. A, unenhanced CT scan from *Case 3* demonstrating infarction involving the inferior and middle temporal gyri on the right. B, higher section from *Case 3* demonstrating infarction of the posterior portion of the middle temporal gyrus.

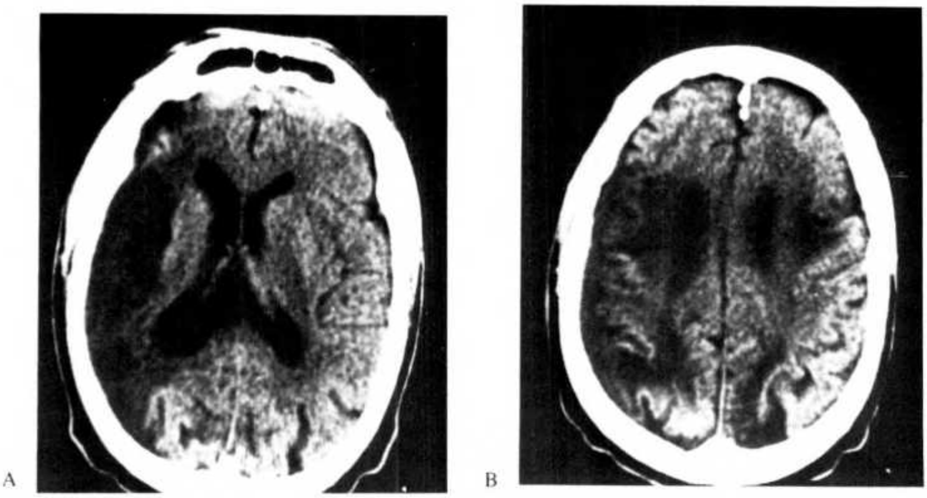


FIG. 4. A, unenhanced CT scan from *Case 4* demonstrating extensive right hemisphere infarction involving the inferior frontal, precentral, superior, middle, and inferior temporal gyri. B, higher section from *Case 4* demonstrating the infarction described in A but also showing involvement of the angular and middle temporal gyri on the right. Small infarctions on the left involving the superior occipital lobe and precentral and postcentral cortex and underlying white matter are also demonstrated.

and posterior parietal infarct. C2 was a 51-yr-old male with a dense right homonymous hemianopia secondary to a left occipitoparietal infarct. C3 was a 56-yr-old male with a left hemiplegia secondary to a right frontoparietal infarct. C4 was a 64-yr-old male with a mild right hemiparesis and conduction aphasia secondary to a left frontoparietal infarct. C5 was a 63-yr-old male with pure alexia and a right homonymous hemianopia secondary to a left occipital infarct.

METHODS

Subjects were asked to bisect a series of lines presented in left and right hemispace. The paradigm differed from most line bisection tasks, however, in that a video camera and monitor were used to dissociate the effects of the hemispace in which the line was viewed from the hemispace in which the line was bisected.

Bold black lines centred on a 11 × 14 inch sheet of white paper served as stimuli. For Case 1, the lines were 5, 10, 20 or 30 cm in length, and for all other subjects the lines were 8, 16, 24 or 32 cm in length. (The line lengths differed for Case 1 because he was investigated before the development of a battery of tests for unilateral neglect.) Stimuli were presented in blocks of 16 trials in which 4 lines of each of the 4 different lengths were presented in random order.

Subjects sat at a table on which a plywood screen was placed directly in front of them parallel to the coronal plane. The screen was 26 cm in height and was placed 10 cm above the table. A 10 cm curtain was suspended from the bottom of the screen to the surface of the table to prevent subjects from looking below the edge of the screen. Stimuli were presented with the medial edge of the stimulus sheet in the subjects' midline. Subjects were instructed to face straight ahead throughout while bisecting lines; compliance was monitored by one of the investigators. When necessary, subjects' heads were held in place by one of the investigators.

As the opaque screen prevented direct visualization of the stimuli, visual feedback to guide line bisection was provided by means of a video camera suspended approximately 120 cm directly over the stimulus and a 63 cm (diagonal) high resolution colour monitor. The monitor was situated approximately 80 cm from the subjects' eyes, and the centre of the monitor was displaced 40 cm laterally from the subjects' midline; the centre of the lines was thus displaced approximately 30° from the midsagittal plane. The camera was positioned so that the stimulus was presented in the centre of the screen, and the line length depicted on the screen was equal to the line length on the stimulus sheet.

As both the video camera and monitor were freely moveable, the hemispaces of line bisection and vision were readily dissociated. Thus subjects were tested in four conditions: line bisection and visual feedback in right hemispace, line bisection and visual feedback in left hemispace, line bisection in right and visual feedback in left hemispace, and line bisection in left and visual feedback in right hemispace.

Case 1 was also tested in a number of additional conditions as part of an extensive investigation of the determinants of head and body position on line bisection (Coslett *et al* , 1984). He was asked to bisect lines placed directly in front of him but with visual feedback from a monitor situated either in left or right hemispace and to bisect lines placed in right or left hemispace but with visual feedback from a monitor in his midline.

Stimulus presentation was blocked so that 16 stimuli were bisected in each of the four conditions. Each of the four conditions were tested in a random sequence in the same session. Cases 1 and 4 were tested in four sessions for a total of 64 line bisections in each of the four conditions; Cases 2 and 3 were tested in two sessions for a total of 32 line bisections in each of the four conditions. All controls were tested with one block of 16 trials in each condition.

RESULTS

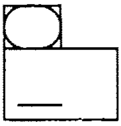
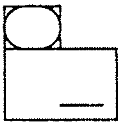
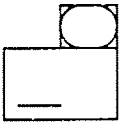
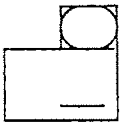
For each trial, the distance from the subject's mark to the midpoint of the line was measured in mm. Deviations to the right were recorded as positive and deviations to the left as negative numbers. One subject sometimes produced responses that were lateral to the end of the line. On those trials, the distance from the middle to the end of the line was used as the error score; for example, if a subject responded at a point 10 cm to the left of the end of a 32 cm line, the error was recorded as 16 cm. Although this scoring procedure substantially underestimated the magnitude of the error on these trials, this method was adopted because the subject's response was often lateral to the end of the response sheet and, consequently, a precise error score could not be determined.

For each subject, the mean error and SD were calculated for each of the four conditions. These results are presented in diagrammatic form for each subject in the Table.

For Cases 1−3, the main effect of hemispace of line bisection (motor action) was assessed by collapsing across the variable hemispace of visual feedback. Thus to assess the effects of the hemispace in which the line was bisected, data from the conditions with line bisection in left hemispace but visual feedback in both right and left visual fields were pooled and contrasted with the data from the conditions with line bisection in right hemispace but visual feedback in both right and left visual fields. The main effect of hemispace of visual feedback was assessed by collapsing across the variable of hemispace of line bisection.

Case 1 erred to the right an average of 35.7 ± 17.0 mm when bisecting in left and 2.4 ± 10.7 mm when bisecting in right hemispace; thus he was significantly more accurate bisecting lines in right as compared to left hemispace ($t = 15.2$, $P < 0.001$). He was also significantly affected by the hemispace of visual feedback ($t = 4.67$, $P < 0.01$); he erred to the right an average of 12.2 ± 9.7 mm on lines presented in left and 25.8 ± 17.2 mm on lines presented in right hemispace. This effect, however, is the

TABLE MEAN ERROR (IN mm) AS A FUNCTION OF EXPERIMENTAL CONDITION

				
Cases				
1	21.7±12.2	2.7±10.7	49.6±20.7	2.0±12.7
2	18.5±10.6	−5.0±3.3	15.1±5.8	−1.7±1.4
3	22.9±31.1	21.2±24.9	9.3±21.5	0.1±24.9
4	19.4±16.6	−55.7±46.4	24.8±18.9	0.6±14.7
Control subjects				
C1	3.7±1.8	2.4±2.5	2.8±2.8	1.4±0.8
C2	−3.8±2.5	−1.8±1.6	−1.7±1.6	−4.7±1.6
C3	−2.2±0.8	0.1±0.7	−6.2±1.1	5.2±2.6
C4	1.7±0.9	1.8±0.8	1.2±0.3	1.2±0.3
C5	1.7±2.0	2.8±1.7	2.1±2.4	3.2±2.1

direction opposite to that predicted by the hemisensory hypotheses and appears to be attributable to the striking impairment noted in the bisect left/view right condition.

A similar pattern of performance was observed in the conditions involving bisection of the line or visual feedback in the midline. When bisecting lines placed directly in front of him, he was not influenced by the hemispace of visual feedback; he erred to the right an average of 6.9 ± 7.7 and 8.4 ± 5.4 mm with visual feedback in left and right hemispace, respectively. In contrast, with visual feedback in the midline he erred to the right an average of 28.3 ± 19.9 mm and 3.5 ± 9.0 mm when bisecting lines in left and right hemispace, respectively.

Case 2 erred an average of 16.8 ± 8.5 mm when bisecting in left and −3.4 ± 2.5 mm when bisecting in right hemispace; he was thus significantly more accurate when bisecting in right hemispace (t = 12.87, $P < 0.001$). He erred an average of 6.8 ± 7.8 mm bisecting lines shown by the monitor in left and 6.7 ± 4.2 mm bisecting lines in right hemispace. This difference was not significant (t = 0.06).

Case 3 erred an average of 16.1 ± 26.7 mm bisecting in left and 10.7 ± 24.9 mm bisecting in right hemispace; this difference was not significant (t = 1.12). This subject, however, did exhibit a significant effect of the hemispace of visual feedback (t = 2.69, $P < 0.01$). She erred an average of 22.1 ± 28.2 mm with visual feedback in left and 4.7 ± 23.3 mm with visual feedback in right.

Case 4 exhibited a pattern of performance that was qualitatively different from Cases 1−3. Like previously reported subjects with neglect (Heilman and Valenstein, 1979), he consistently erred to the right and was more accurate in right (7.3 ± 5.9 mm) as compared with left hemispace (33.4 ± 18.2 mm) on the line bisection task (t = 7.72, $P < 0.001$) with stimuli presented free-field. When the hemispaces of visual feedback and line bisection were dissociated, however, his performance was markedly altered. When bisecting lines in right hemispace but receiving visual feedback in left hemispace, for example, he placed his mark to the left of the entire line in more than half of the trials. Using the conservative scoring procedure described above, his mean error to the left in this condition was 55.0 mm. The subject was not confused during this testing; he was cognisant of the fact that he consistently deviated to the left in this condition but, despite verbal self-admonishments, was unable to overcome the tendency to err to the left. His performance was not influenced by stimulus length.

Because of the large magnitude of the error in this condition, the analysis performed for Cases 1 – 3 was considered inappropriate for Case 4. To test the hypothesis derived from these and other data (Coslett, unpublished results) that the subject's performance on the line bisection task was strongly influenced by the hemispace of visual feedback, an additional investigation was performed. He was asked to bisect lines presented in the midline with visual feedback in either right or left hemispace. There were 32 trials in each condition. He erred an average of 31.3 mm to the *left* with the visual feedback in left and 11.8 mm to the *right* with visual feedback in right hemispace. Thus, like Case 3, his performance was strongly influenced by the hemispace from which visual feedback was provided.

Data from the control subjects are also provided in the Table. No control subject exhibited a significant effect of the hemispace of line bisection or visual feedback.

DISCUSSION

The results of this investigation support both the directional hypokinesia and hemisensory attention-representation deficit theories. As predicted by the former hypothesis, Cases 1 and 2 bisected lines significantly more accurately in right as compared with left hemispace. The performance of Case 3, in contrast, was in accord with the prediction of the hemisensory attention-representation deficit hypothesis as she bisected lines significantly more accurately when visual feedback was provided in right as compared with left hemispace. The performance of Case 4 was also consistent with the hypothesis that neglect may be attributable to a disruption of sensory processing.

Although unfortunately not available for all subjects, data from the intermediate conditions with midline visual feedback and lateralized line bisection, as well as midline line bisection and lateralized visual feedback, are also of interest. For Case 1, these data support the contention that the subject's performance was influenced by the hemispace of line bisection but not the hemispace of visual feedback. Similarly, for Case 4, the data from the condition in which lines were bisected in the midline but visual feedback provided in right or left hemispace support the argument that the subject was influenced by the hemispace in which the visual feedback was provided.

The performance of Cases 1 and 4 is also noteworthy in another respect. When bisecting lines in left hemispace, visual feedback from the right hemispace did not improve their performance relative to the condition with visual feedback from the left, but instead appeared to make their error towards the right more severe. Case 4 also showed this effect in the opposite direction. To our knowledge such an effect has not previously been reported. One possible reason for this effect is that attentional and intentional systems may be linked such that orienting in one direction or to one location introduces a bias to act in the same direction or location. Normal subjects as well as some brain-damaged patients are able to overcome or inhibit this bias; Cases 1 and 4 had difficulty inhibiting this bias.

Several lines of evidence suggest that the results reported here are not artefacts related to the experimental paradigm. First, the brain-injured control subjects performed well in all experimental conditions. Secondly, the performance of the experimental subjects on the typical line bisection task with direct visualization was, for the most part, quite similar to performance in the corresponding experimental conditions (i.e., line bisection and visual feedback in the same hemispace). Additionally, as several control subjects had visual field deficits but performed well on the task, the impaired performance of the subjects with neglect cannot be attributed to visual field deficits alone.

These data have theoretical implications. The demonstration that some subjects with unilateral neglect are significantly affected by the hemispace in which the line is bisected but not the source of visual feedback would appear to be inconsistent with a 'representational' defect hypothesis that attributes neglect to a failure to generate an accurate internal map marking the location of external stimuli. Although we agree with Bisiach and colleagues (1978, 1979) that neglect may, in some instances, be attributed to a disruption of an internal 'representation'—the data from Cases 3 and 4 may be interpreted in this manner—it is not clear how a failure to construct an accurate internal representation would account for the performance of Cases 1 and 2 who performed poorly in left hemispace even with visual input into the intact left hemispace.

The accounts of neglect offered by Riddoch and Humphreys (1983) as well as Heilman and Valenstein (1979) are subject to a similar criticism. Heilman and Valenstein (1979) and Riddoch and Humphreys (1983) both gave subjects with left-sided neglect lines to bisect in right and left hemispace. They also gave visual cues on the left and right sides of the line. Although Heilman and Valenstein (1979) found that it was the hemispace of bisection that was critical to performance, Riddoch and Humphreys (1983) found it was the attentional cues. The suggestion of Riddoch and Humphreys (1983) that subjects may fail to orient attention automatically to left hemispace does not explain the performance of Cases 1 and 2, whose performance was significantly influenced by the hemispace in which the stimuli were presented. Similarly, the explanation of Heilman and Valenstein (1979) that there is an impairment in premotor processing, that is, a directional hypokinesia, cannot account for the performance of Case 3. The demonstration that different types of processing impairments may underlie clinically indistinguishable behavioural deficits strongly supports the contention that the neglect syndrome may be fractionated (*see also* Bisaich *et al.*, 1983).

An additional point of interest concerns the anatomical substrate of the performance exhibited by our patients. Electrophysiological investigations have suggested that the inferior and posterior parietal cortex may be critical for monitoring and directing attention within the visual environment (Mountcastle *et al.*, 1975; Robinson *et al.*, 1978). Additionally, this cortical region may be critical for the representation of spatial location; Andersen and colleagues (Andersen and Mountcastle, 1983; Andersen *et al.*, 1985), for example, have suggested that the posterior parietal neurons code spatial information in an egocentric rather than retinotopic coordinate system; as retinotopically coded representations are altered with each eye movement, the egocentrically coded spatial information registered by posterior parietal neurons might be expected to represent spatial information in a manner appropriate for articulation with motor and nonvisual sensory inputs. In light of these data, those subjects demonstrating an impairment in the use of visual information presented on the left might be expected to have lesions involving the posterior and inferior parietal cortex. The infarct in Case 3 did, indeed, involve these areas as well as posterior temporal cortex. Although the large right hemisphere infarct as well as the left hemisphere lesions in Case 4 render anatomical-behavioural correlations difficult, it should be noted that the posterior temporal and posterior and inferior parietal areas were also infarcted in this subject. Furthermore, these regions appeared to be at least relatively preserved in Cases 1 and 2. One goal of further research will be to specify the levels of sensory and motor processing at which impairments may cause the clinical syndrome of neglect and to correlate these with the anatomical substrate.

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OPTIC NEURITIS: VARIATIONS IN TEMPORAL MODULATION SENSITIVITY WITH RETINAL ECCENTRICITY

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SUMMARY

Temporal modulation sensitivity functions were measured centrally and at eccentricities of 2.5°, 5° and 10° in the temporal visual field of 12 patients with recovered optic neuritis and in a group of matched normal controls A circular, spatially uniform stimulus of 1° angular subtense was presented with sinusoidal modulation at 5, 8, 14 and 23 Hz. The general pattern of results in patients was a loss of sensitivity relative to normal controls at all temporal frequencies at 0° and 2.5° eccentricity, with rather greater losses occurring at the medium-to-lower temporal frequencies. At 5° eccentricity, the losses were confined to medium temporal frequencies only, and at 10° eccentricity there was no significant loss at any temporal frequency. These findings may be explained by a greater vulnerability of optic nerve fibres of small diameter to the effects of demyelinating disease.

INTRODUCTION

Demyelination of nerve fibres leads to delayed transmission of nerve impulses, an increased refractory period, and the possibility of conduction block if demyelination is sufficiently severe (Halliday and McDonald, 1977). As a consequence, an impairment of temporal visual function is frequently evident in multiple sclerosis (MS) and recovered optic neuritis (ON).

A traditional method of assessing altered temporal response function is by measurement of the critical flicker frequency (CFF), which is often lowered in MS patients (Titcombe and Willison, 1961; Daley *et al.*, 1979; Mason *et al.*, 1982). The disadvantage of this method is that it assesses sensitivity at a single high temporal frequency. A more comprehensive approach is to measure the full de Lange attenuation characteristic (de Lange, 1952, 1958). This method employs a stimulus whose luminance varies sinusoidally with time, but rather than the temporal frequency being adjusted to threshold so that flicker is just detectable, as in CFF determinations, the frequency is held constant and

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the modulation depth is adjusted to threshold. Modulation depth is specified in terms of the time-varying luminance L as $(L_{max}-L_{min})/(L_{max}+L_{min})$. The threshold may be defined as the modulation depth at which flicker is detected in a certain proportion (e.g., 50%) of trials. Threshold measurements are made at a number of temporal frequencies to yield the attenuation characteristic, a direct temporal analogue of the contrast sensitivity function used to assess spatial vision.

It has been commonly assumed that the overall spatial response of the visual system may be determined by a collection of discrete 'channels', each channel sensitive to a narrow band of spatial frequencies (Pantle and Sekuler, 1968; Blakemore and Campbell, 1969). Similarly, it has been suggested (Kulikowski and Tolhurst, 1973; Watson and Robson, 1981; Hess and Plant, 1985) that the temporal response of the visual system may be determined by a collection of temporal channels, the relative activities of which depend on the spatial frequency of the stimulus. Hess and Plant (1985) proposed that, at medium-to-high spatial frequencies, two channels mediated the overall response, one being low-pass for temporal frequency and the other band-pass, whereas at low spatial frequencies there was a third channel that was high-pass for temporal frequency.

Plant and Hess (1985), using sinusoidal gratings of fixed spatial frequency, examined the effect of ON and MS on the de Lange attenuation characteristic and, although a number of different patterns of loss were evident, the most common was one consistent with a greater loss of function of the low-temporal-frequency (low-pass) channel. Medjbeur and Tulunay-Keesey (1985), in a related investigation, studied the effect of temporal modulation on the contrast sensitivity function in patients with ON or MS. From their results they suggested that two temporal-frequency channels determined the overall temporal response and that it was the lower temporal frequency ('sustained') channel that was more affected in ON and MS.

One of the consequences of using sinewave gratings is that they involve extended areas of the retina and therefore may not reveal highly localized variations in performance that are associated with patchy demyelination of the optic nerve. Another important factor is the possible association between the effects of demyelination and the diameters of optic nerve fibres (discussed in more detail later). There is a natural variation in fibre diameter with increasing retinal eccentricity, the highest concentration of small diameter fibres serving the macula (Potts *et al.*, 1972; Sanchez *et al.*, 1986). Any losses associated with a particular range of fibre diameters may be less evident with the use of a large stimulus such as a sinewave grating. In the present investigation a small (1° angular subtense), spatially uniform, circular (spot) stimulus was used to measure de Lange attenuation characteristics at the central fovea and at three increasingly eccentric sites in the temporal visual field of 12 patients with previous ON. For comparison, the same sites were tested in a group of 8 matched normal controls. Although the spot stimulus used was, in spatial-frequency terms, broad band, its small spatial size made possible a test of the relationship between temporal modulation sensitivity loss and retinal location in recovered ON.

METHODS

Subjects

Twelve patients were investigated (1 male, 11 females). All had previous ON and, in some cases, evidence of demyelination elsewhere in the central nervous system. None were in an acute phase of the disease at the time of testing and none had nystagmus. Patients were classified according to the criteria of McDonald and Halliday (1977), with isolated ON accepted as an additional diagnostic category. Clinical details are given in Table 1. Eight healthy subjects (1 male, 7 females) with a similar age range to the patients acted as normal controls. No control subject had an acuity worse than Snellen 6/9. Two of the controls were experienced psychophysical observers; the remainder were experimentally naive. Subjects wore their own spectacles (or, if necessary, trial lenses were substituted). Informed consent was obtained from each patient and control subject.

Stimuli and apparatus

The stimulus consisted of a temporally sinusoidally modulated, spatially uniform, yellow circular field, of 1° angular subtense with a contiguous, steady, uniform annular surround (5° outer diameter) matched in colour and luminance to the time-averaged (d.c.) stimulus (a configuration that has been shown to maximize sensitivity to achromatic flicker; Wisowaty, 1981). The luminance of the time-averaged stimulus and surround was $2.0 \log_{10} \mathrm{cd \cdot m^{-2}}$, peak-emission wavelength 584 nm. Stimulus duration was 2 s with, at the beginning and end of the stimulus interval, an additional 0.5 s ramp-up and ramp-down of modulation depth. The stimuli were generated within a special-purpose perimeter system. The combined output from 2 identical light-emitting diodes (LEDs) (Marl BP200 UBA 12H CW) under the control of a digital electronics system was used to produce the stimulus, with 8 other LEDs (Marl BP200 FUA 12H CW), identical apart from lens configuration, used to produce the surround field. A high-frequency pulse train was used to drive the stimulus LEDs (minimum frequency 800 Hz). Sinusoidal modulation of the pulse density produced the apparent sinusoidal amplitude modulation of the stimulus luminance. The whole system was under the control of a laboratory microcomputer. Harmonic distortion in the stimulus was assessed with a Bruel and Kjaer frequency analyser; the amplitude of harmonics of frequencies up to 500 Hz was found to be more than 50 dB below the amplitude of the fundamental.

The stimulus and surround were presented on a large steady background field (40° W×24° H) of luminance $1.0 \log \mathrm{cd \cdot m^{-2}}$, which maintained a constant state of retinal adaptation. Stimuli were viewed monocularly and stimulus presentation was initiated by the subject using a push-button box connected to the computer.

TABLE 1 SUMMARY OF CLINICAL DATA*

Case	*Age (yrs)*	*Sex*	*Snellen acuity*	*Near vision acuity*	*Optic disc pallor*	*Visual field*	*Classification*	*Duration (yrs)*	*No. of attacks*	*Optic neuritis*	*Lesions at other sites*
1	38	F	6/6	N5	+	+	Clinically definite	6	5	+	Spinal cord, brainstem
2	30	F	6/12	N5	+	+	Clinically definite	4	4	+	Brainstem
3	43	F	6/18	N9	+	+	Clinically definite	5	5	+	Brainstem, spinal cord
4	36	F	6/9	N4.5	+	+	Early probable	0.6	2	+	Brainstem
5	39	F	6/9	N5	+	+	Optic neuritis	1.4	1	+	
6	39	F	6/6	N4.5	+	+	Optic neuritis	6	1	+	
7	37	F	6/9	N5	+	+	Clinically definite	2	2	+	Spinal cord
8	39	F	6/6	N4.5	–	–	Clinically definite	6	2	+	Spinal cord
9	40	M	6/5	N5	–	+	Clinically definite	11	9	+	Brainstem, spinal cord
10	35	F	6/12	N5	+	+	Clinically definite	11	4	+	Brainstem
11	30	F	6/6	N4.5	+	–	Optic neuritis	0.7	1	+	
12	37	F	6/5	N4.5	–	–	Optic neuritis	0.8	1	+	

* Optic disc pallor, the presence of scotomata in the visual field, and optic neuritis are indicated by + in the appropriate columns

Procedure

Measurements were made at 4 retinal sites (central, 2.5°, 5° and 10° eccentricity in the temporal visual field), with 4 temporal frequencies being tested at each site (5, 8, 14 and 23 Hz). For each participant in this study it was possible to record modulation thresholds at all these sites and frequencies.

The subject sat in a chair of adjustable height and placed his or her eye against the rubber eye-cup of the viewing system. An artificial pupil was not used. The subject fixated the stimulus for central presentation or an off-axis red (LED) fixation target for eccentric presentation. At each site, measurements were made in ascending order of temporal frequency. There were 30 stimulus trials (with 5 empty 'catch' trials randomly interleaved) at each frequency, and in each trial the subject reported (forced-choice) whether or not the stimulus was seen to flicker. The level of the stimulus modulation depth in each trial was determined by a modified PEST routine (Taylor and Creelman, 1967; Hall, 1981) that produced a spread of levels sufficient to define a sigmoidal 'frequency-of-seeing' curve. There was a short (5 s) break after 15 trials and a break of at least 2 min between each new frequency setting. The 4 sites were tested in separate counterbalanced sessions over 2 days, with 0° and 5° (in that order) examined on the first day, and 10° and 2.5° (also in that order) examined on the second day.

Visual fields for each subject were plotted using a 2 m Bjerrum screen.

Data analysis

A computer-based technique (Foster, 1986*a*) was used to analyse the data. A cumulative Gaussian curve was fitted to each set of frequency-of-seeing data by a maximum-likelihood procedure. Threshold was defined as the value of modulation depth corresponding to 50% detection. The SD of this threshold was estimated by a bootstrap technique (Foster and Bischof, 1987). Because there were significant deviations from normality in the distribution of patient thresholds, a conservative nonparametric approach was adopted to the analysis. Mann-Whitney U tests were used to assess the significance of differences in threshold between normal and patient groups and the significance of the trend in these differences over temporal frequency and eccentricity. In all cases, two-tailed tests were used to make comparisons.

RESULTS

Fig. 1 shows, at each eccentricity, threshold modulation depth as a function of temporal frequency. Thresholds for patients are plotted as individual data points and for normal controls as group means connected by solid lines, with the hatched area indicating ±2 SD.

The results of the analysis of the difference between the patient and control groups at each frequency and eccentricity are shown in Table 2. At 0° and 2.5° eccentricity there was a significant difference in temporal modulation sensitivity between the patient and control groups at all frequencies, with rather greater losses occurring at the medium-to-lower temporal frequencies. At 5° eccentricity, the losses occurred at medium temporal frequencies only, and at 10° eccentricity there was no significant loss at any temporal frequency. A nonparametric test of the eccentricity dependence of the losses, evaluated over all temporal frequencies, showed a significant linear trend in the improvement with eccentricity ($z = -2.30$, $P < 0.02$, two-tailed test).

Measurement of visual fields revealed scotomata within the test region in only 3 patients. Case 2 had a central scotoma extending to 10° eccentricity and Case 5 had a relative central scotoma, also extending to 10° eccentricity. Case 4 had a relative paracentral scotoma extending from 2.5° to 10° eccentricity. Temporal-modulation sensitivity losses for these patients were as follows. Case 2 showed significant losses over all frequencies

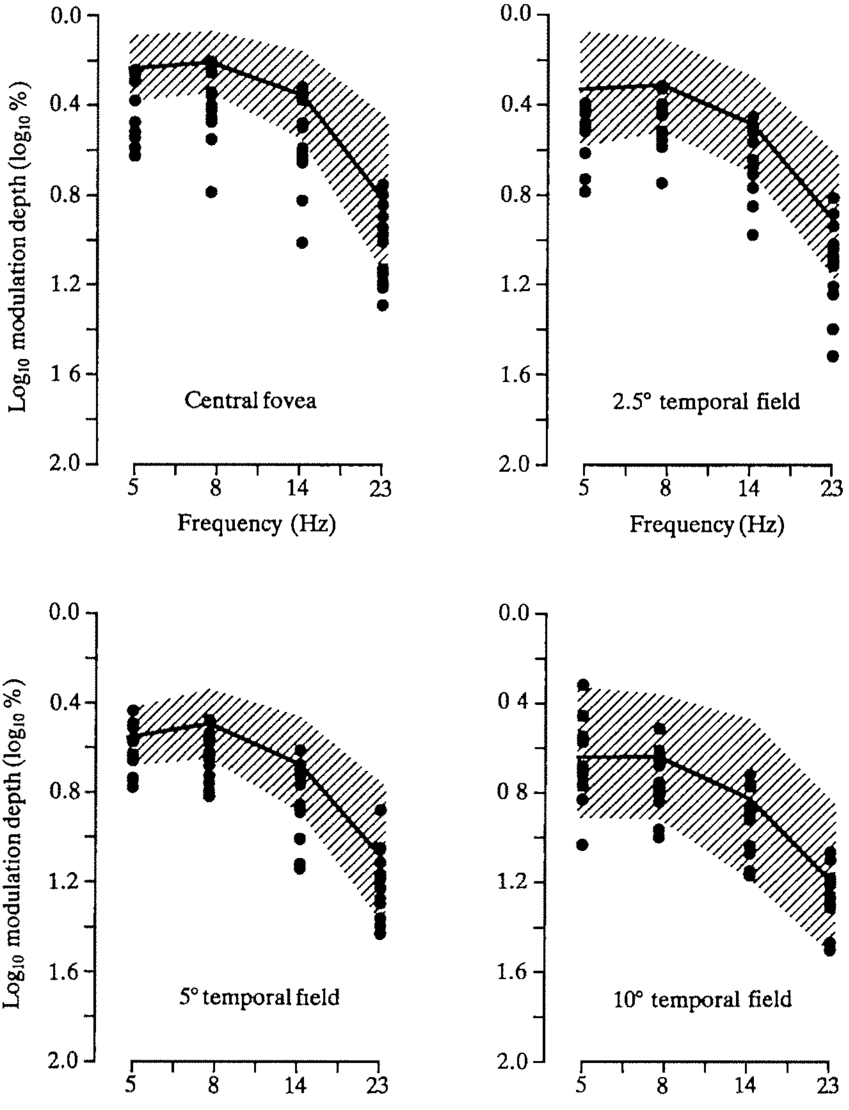

FIG 1 Modulation sensitivity as a function of temporal frequency (de Lange attenuation characteristic) at 4 retinal eccentricities for patients with previous optic neuritis and for normal controls Data points represent individual values for patients and the solid lines connect means for the control group, with ±2 SD shown by the hatched areas.

at 0°, but with increasing eccentricity these losses diminished, more rapidly at medium-to-high frequencies. Case 4 showed significant losses over all frequencies at both 0° and 2.5° eccentricities, and these losses also decreased with eccentricity. For Case 5, losses were confined to medium and high frequencies (8–23 Hz) and, at the higher frequencies, these losses also decreased with eccentricity.

TABLE 2. SUMMARY OF SIGNIFICANT DIFFERENCES IN MODULATION SENSITIVITY BETWEEN THE PATIENT AND CONTROL GROUPS AS A FUNCTION OF STIMULUS ECCENTRICITY AND TEMPORAL FREQUENCY

		Frequency (Hz)			
		5	*8*	*14*	*23*
Retinal eccentricity	0°	++	++	+	+
	2.5°	+++	+++	+	+
	5°	−	+++	+	−
	10°	−	−	−	−

(− = n s., + = $P < 0.05$, ++ = $P < 0.02$, +++ = $P < 0.01$, two-tailed tests)

DISCUSSION

The results of this investigation clearly indicate systematic variations in the pattern of temporal-modulation sensitivity loss with retinal location in recovered optic neuritis. There was a general loss relative to normal controls at all temporal frequencies at 0° and 2.5° eccentricity, a loss restricted to medium temporal frequencies at 5° eccentricity, and no loss at any frequency at 10° eccentricity. These effects are compatible with the results of some animal models of demyelinating disease. Morphological studies (Tansey *et al.*, 1985) of fibres in the optic nerves of mice infected with Semliki Forest virus have suggested that smaller diameter fibres were more likely to be affected by demyelination. Because the average diameter of fibres is greater outside the fovea (Potts *et al.*, 1972; Sanchez *et al.*, 1986) and because these fibres are certainly capable of transmitting signals at higher temporal frequencies (Cleland and Levick, 1974; de Monasterio, 1978), the relative preservation in patients of high temporal-frequency modulation sensitivity at intermediate eccentricities (2.5° − 5°) and the preservation of sensitivity at all frequencies at larger eccentricities could be due to conduction by these less affected fibres.

A loss of function of small diameter fibres is consistent with the results of a study by Trauzettel-Klosinski and Aulhorn (1987) in which patients made brightness matches of a steady field to a flickering test field. The reduction in brightness at medium (5 − 15 Hz) temporal frequencies was concluded to be the result of selective damage to X-type fibres. It is not necessary to suppose that a particular temporal frequency channel, or fibre of a particular type, or particular retinal area is directly and preferentially affected in ON and MS (Foster *et al.*, 1985), merely that conduction along small diameter nerve fibres is, because of their physical characteristics, more vulnerable to disruption by demyelinating lesions.

Although a particular psychophysical channel may not be directly affected in ON and MS, a psychophysical channel that was low pass (Plant and Hess, 1985; Medjbeur and Tulunay-Keesey, 1985), and that depended upon impulse transmission along small diameter fibres, might exhibit altered temporal modulation sensitivity. This alteration could result (1) from a decrease in the overall sensitivity of the channel as a consequence of conduction block reducing the number of active fibres serving a given area of the retina, and (2) from a change in the shape of the temporal response characteristics, which could become increasingly low-pass as transmission of high frequency impulses was impaired. The effect of the second mechanism would be to modify the overall attenuation characteristic so that there was a loss in sensitivity at medium temporal frequencies, as was found in this study. Given the general increase in fibre diameter with increase in retinal eccentricity, the effect of demyelination would be greatest in the fovea where, it seems likely, the responses of all psychophysical channels are mediated by smaller diameter fibres (Potts *et al.*, 1972; Perry *et al.*, 1984; Sanchez *et al.*, 1986; Cowey *et al.*, 1989). In this region, losses in temporal modulation sensitivity should extend over a greater range of temporal frequencies, as was also found here.

Classically, localized visual sensitivity loss has been assessed by kinetic perimetry. Visual field measurements of patients during acute ON showed central scotomata to be present in the large majority of cases (Nettleship, 1884; Hyllested and Møller, 1961). In recovered ON, however, the proportion of patients with central scotomata is reduced. Perkin and Rose (1979) found that one-third of patients with recovered ON had evidence of a central scotoma, whereas Patterson and Heron (1980) in a study of 54 patients with MS or recovered ON found the commonest visual field defect to be an arcuate scotoma.

A number of studies have used different techniques to examine the variation of temporal responsiveness with retinal eccentricity in patients with ON and MS. These studies include those of Miles (1951) who measured the variation of CFF over the visual field, Galvin *et al.* (1976) who measured two-flash resolution and relative perceptual latency, and Brussell *et al.* (1981/82) who used a multiflash campimetry technique (altering the duty-cycle of a light flickering at 5 Hz until flicker could just be detected) to measure temporal resolution. A common finding in these studies was of 'islands' of poorer performance in the visual fields of patients, although these islands were not necessarily correlated over different temporal functions (Galvin *et al.*, 1976; Snelgar *et al.*, 1985), or with obvious retinal structures. It does not follow that these patchy losses need be directly related to more systematic losses in temporal modulation sensitivity with retinal eccentricity. In ON, demyelination may affect the full thickness of the nerve (Ulrich and Groebke-Lorenz, 1983) but, because of the topography of plaques (Fog, 1965), the length over which demyelination affects transmission will vary over the nerve section. The only data in the present study bearing on a possible relationship between field losses and eccentricity-dependent changes in temporal modulation sensitivity come from the 3 patients who had field defects within the test region. Two had scotomata extending from the fovea to 10° eccentricity and the third had a paracentral scotoma extending from 2.5° to 10° eccentricity. Each of these 3 patients showed losses in modulation sensitivity that diminished with eccentricity, most rapidly at medium-to-high temporal frequencies.

If, as suggested, the results of the present investigation may be attributed to a greater effect of demyelination on small diameter fibres, then it might be expected that patients with ON and MS would show losses of spatial contrast sensitivity that also diminished with eccentricity. Contrast sensitivity determined with high spatial-frequency gratings, thus assessing acuity, should be most affected. Plant and Hess (1987) used circular patches of sinewave gratings (2.5° in diameter) counterphase sinusoidally modulated at 8 Hz to measure threshold contrast sensitivity at a number of eccentricities, up to 7.5°, within the central visual field of patients with recovered ON. For medium-to-low spatial frequencies, they found no variation in sensitivity, relative to control subjects, over this region of the field. When spatial-frequency performance was extrapolated, however, to yield estimated acuity losses, they found that at the largest eccentricities there was a significant reduction in the impairment. A factor contributing to the absence of eccentricity-dependent losses at lower spatial frequencies may have been the choice of temporal frequency of modulation. In the present measurements, the threshold deficit was found to vary most rapidly with eccentricity at the lowest temporal frequency of 5 Hz.

An effect of demyelination related to fibre size may also have implications for variations in colour vision in ON and MS. There is evidence that chromatic function is mediated by smaller diameter fibres that project to the parvocellular layers of the lateral geniculate nucleus (Perry *et al.*, 1984; Livingstone and Hubel, 1987). If smaller diameter fibres are more affected by demyelination, then colour vision losses should be greatest in the fovea and should diminish with eccentricity, a prediction which is consistent with common reports in ON of a central or relative central scotoma with an associated loss in colour perception (Gunn, 1897; Burde and Gallin, 1975; Glaser, 1976). Whether losses in chromatic sensitivity are observed to be greater than losses in luminance sensitivity depends on a number of factors. With small (0.25°) centrally located stimulus fields, chromatic losses have been found to be indistinguishable from luminance losses (Foster *et al.*, 1985), both systems there presumably being subserved by fibres with similar small diameters (*see* Travis and Thompson, 1989, for related arguments). With medium-to-large stimulus fields (2°–6.5°), where there is more scope for variation in fibre diameter spectra and the possibility of obtaining responses at lower spatial frequencies, greater losses in chromatic sensitivity have been recorded (Fallowfield and Krauskopf, 1984; Mullen and Plant, 1986). Discussion of some of the technical issues has been given in Foster (1986*b*) and Plant (1990).

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HISTOMETRIC CHARACTERISTICS AND REGENERATIVE CAPACITY IN WOBBLER MOUSE MOTOR NEURON DISEASE

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SUMMARY

In wobbler mice, histometric features and the response to injury in the less affected hindlimb nerves were compared with the severely affected forelimb nerves which were studied previously. The frequency of vacuolated anterior horn cells was not different at the individual ages studied (3 and 6 weeks, 3 months), but with the three ages combined, the frequency in the two systems differed significantly, being 1.7±0.9% in the cervical and 0.6±0.7% in the lumbar anterior horns ($P = 0.005$). The number of large myelinated nerve fibres (≥ 6 μm in diameter) became progressively reduced in ventral roots as the disease advanced, particularly for the forelimb. The total number of myelinated axons was well preserved in the hindlimb ventral roots because of an increase in the number of small myelinated nerve fibres. The regenerative capacity of motor neurons after axotomy (tested by radiolabelling growth cones) was well preserved. In the hindlimb, the increased numbers of small myelinated fibres in ventral roots and the normal regenerative capacity in the sciatic nerve and its branches contrast with the severely affected forelimb system. Our studies suggest that cervical and lumbar anterior horn cells have different susceptibilities to the wobbler gene, producing vulnerability in one system and disease resistance in the other.

INTRODUCTION

In the motor neuron diseases, certain groups of motor neurons are affected while others are spared. In amyotrophic lateral sclerosis (ALS), for example, ocular motor neurons (Weiner, 1980) and sacral motor neurons of Onuf's nucleus (Mannen *et al.*, 1977; Iwata and Hirano, 1978) are relatively spared, whereas bulbar motor neurons are often severely affected (Norris *et al.*, 1979). Furthermore, the disease affects only upper motor neurons in some patients and lower motor neurons in others (Charcot, 1881; Rowland, 1982). In Werdnig-Hoffmann disease, phrenic motor neurons are spared (Kuzuhara and Chou, 1981). In chronic spinal muscular atrophies, the various forms are differentiated clinically in terms of selective motor involvement (Rowland, 1982). The basis for selective neuronal involvement has not been well analysed and may be important for the understanding of motor neuron disease.

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Hereditary motor neuron disease in the wobbler mouse is characterized by perikaryal vacuolar degeneration of anterior horn cells and axonal loss (Duchen *et al.*, 1966, 1968; Papapetropoulos and Bradley, 1972; Andrews *et al.*, 1974; Andrews, 1975). These changes produce progressive atrophy, paralysis, and contractures in the forelimb muscles, the hindlimb muscles being spared (Duchen *et al.*, 1968; Mitsumoto and Bradley, 1982; Mitsumoto, 1988). Our previous studies have shown that a severe depletion of large motor nerve fibres in the forelimb system (Mitsumoto and Bradley, 1982, 1985) is associated with impairments of slow axonal transport and axonal regeneration (Mitsumoto, 1985; Mitsumoto and Gambetti, 1986). To characterize the selective neuronal involvement, we have quantified some of the differences between the spared hindlimb system and the affected forelimb system. Some of the findings in unaffected hindlimbs were presented in a recent review (Mitsumoto, 1988).

METHODS

Animals

The animals used in this study were from the wobbler mouse colony at the Cleveland Clinic Research Institute Animal Center. Nerve tissue from affected animals and their normal littermates was examined by histometric methods at 3 wks, 6 wks, and 3 months of age (Mitsumoto and Bradley, 1982; Mitsumoto, 1985; Mitsumoto and Gambetti, 1986). For the study of axonal regeneration, 3-month-old animals were used because they were large enough for assessing axonal regeneration accurately (Mitsumoto, 1985). Halothane anaesthesia was used for all procedures.

Histometric studies

The animals were perfused for 20 min with ice-cold phosphate-buffered 3.6% glutaraldehyde solution at pH 7.4 through an intracardiac catheter. Lumbar spinal cord segments (L5 and L6) and corresponding ventral roots at the dorsal root ganglia were sampled and embedded into Spurr epoxy resin. The tissue preparation was identical to that previously described (Mitsumoto and Bradley, 1982). Semithin sections of C7 and C8 spinal cord which were available from previous studies (Mitsumoto and Bradley, 1982), and those of the lumbar spinal cord prepared in this study, were set aside; one slide was chosen at random per animal. Without the examiner knowing the identification of the slide, the anterior horn region was examined under a × 100 objective lens. The anterior horn cells were classified as either normal or vacuolated, based on previous criteria (Mitsumoto and Bradley, 1982, 1985). The percentage of vacuolated neurons was calculated for each animal.

Cross-sections of L5 and L6 ventral roots were studied in 15 wobbler mice and 18 normal littermates. Histometric studies were performed on the myelinated nerve fibres in photomicrographs at a magnification of ×1000 with a digitizer connected to a Wang computer (Mitsumoto and Gambetti, 1986). The cervical myelinated nerve fibres in a 3-month-old wobbler mouse (and its normal littermate) were recounted by the same method so that we could compare the results of an earlier histometric method with current data.

Axonal regeneration

The right sciatic nerve was crushed twice for 30 s approximately 4 mm below the sciatic notch (20–25 mm from the spinal cord) with watchmakers' forceps (Mitsumoto, 1985) The epineurium at the middle of the crush site was marked with a 10-0 silk suture (Mitsumoto, 1985). We examined the effect of this crush technique on sciatic nerve axons in a pair of animals (1 wobbler and 1 normal littermate) at 7 days after crushing using Bodian's silver method (Mitsumoto, 1985), which selectively stains neurofilaments (Gambetti *et al.*, 1981). As in a previous study, the crush disrupted the endoneurial nerve fibre sheaths while preserving the continuity of the epineurium and perineurium (Seddon, 1943; Mitsumoto, 1985) at the site of the crush; many of the regenerating axons had an irregular intersecting course within fascicles.

To measure axonal outgrowth, we used the fast axonal transport method to label growth cones (Griffin *et al.*, 1976). This radiolabelling technique detects outgrowth distances in consecutive nerve segments as growth cones are labelled at a five-fold greater density than the axons themselves (Griffin *et al.*, 1976). Outgrowth distances were measured at 5 days (9 controls and 5 wobbler mice) and 8 days (9 animals each) after nerve crush. These durations were chosen so that results could be compared with published findings for the forelimb (Mitsumoto, 1985). At 24 h before the mice were killed, L1 and L2 right posterior hemilaminectomy was performed and 0.25 μl of an equal mixture (in radioactivity) of tritiated proline (2923 GBq/mmol [42 Ci/mmol], Amersham, Chicago, IL) and tritiated lysine (1554 GBq/mmol [79 Ci/mmol], Amersham, Chicago, IL), adjusted to 3.7 MBq/μl [100 μCi/μl], was injected stereotactically with a glass micropipette (40–50 μm tip diameter) into four locations in the right L5 and L6 ventral horn. The coordinates for injection were determined by prior experiments using methylene blue injections. Body temperature was maintained by an intermittent heat lamp controlled by a rectal thermocouple. After isotope injection, the surgical area was washed with sterile saline, and the overlying muscles sutured with 7-0 silk. The skin was closed with small Michel clips. Postoperatively, the animals were kept warm on a Braintree thermopad (Braintree, MA); 24 h later (at 5 or 8 days after nerves were crushed), the animals were reanaesthetized and killed by intracardiac perfusion.

After perfusion, the hindlimb nerves were dissected down to the ankle, beginning at the spinal cord with the L5 and L6 ventral roots and including all distal branches. The nerves were gently straightened on a card to restore the original length of the branches. Consecutive 2 mm nerve segments were cut, beginning at the middle of the crush site and extending distally. The first segment was designated as the 'crush site'. The nerve segments were processed for liquid scintillation spectrometry (Mitsumoto, 1985). The maximum outgrowth distance was arbitrarily defined as the point where protein radioactivity had decreased to 10% of the protein radioactivity found in the first segment distal to the crush site (Forman *et al.*, 1981; Mitsumoto, 1985). The rate of outgrowth was calculated by the least-squares method, based on all distances at 5 and 8 days; additional time points were not needed because axonal outgrowth is known to be a linear function of time in small mammals (Forman and Berenberg, 1978; Forman *et al.*, 1979). Differences between wobbler mice and controls were analysed by Student's t test.

RESULTS

Vacuolated neurons in wobbler mice

Between 100 and 120 anterior horn cells were counted in each randomly selected anterior horn region. As shown in Table 1, the cervical region had more vacuolated neurons than the lumbar region. Although the differences were not statistically significant at each age, probably because of the small sample size, the pooled data showed a significant difference.

TABLE 1 VACUOLATED ANTERIOR HORN CELLS IN WOBBLER MICE

	Mean+SD % (n)	
Age	*Cervical*	*Lumbar*
3 wks	2.5±1.0% (5)	0.9±0.7% (4)
6 wks	1.8±0.7% (4)	0.4±0.8% (3)
3 mos	1.1±0.9% (4)	0.3±0.7% (4)
All ages	1.7±0.9% (13)	0.6±0.7% (11)*

* There are significantly fewer vacuolated neurons in the lumbar anterior horn than in the cervical anterior horn, when all ages are combined ($P < 0.005$)

TABLE 2 NUMBER OF SMALL (<6 μm) AND LARGE (≥6 μm) MYELINATED FIBRES IN LUMBAR (L5 AND L6) VENTRAL ROOTS

	Controls (mean ± SEM)			*Wobbler mice (mean ± SEM)*		
Age	*Total*	*Small*	*Large*	*Total*	*Small*	*Large*
3 wks	927±42 (n = 6)	532±46	398±85	821±97 (n = 4)	521±17	290±110
6 wks	946±20 (n = 6)	352±21	627±49	891±15 (n = 6)	479±34[2]	415± 40[3]
3 mos	972±47 (n = 6)	352±14	618±39	810±60 (n = 5)	433±31[1]	433± 53[2]

The total number of myelinated fibres does not diminish significantly in wobbler mouse lumbar ventral roots throughout the disease course. Whereas the numbers of large myelinated fibres are reduced at 6 wks and 3 mos of age, the numbers of small myelinated fibres are clearly increased in wobbler mice. This is in contrast to wobbler mouse forelimb ventral roots, where total myelinated fibre numbers are depleted as the number of small fibres is not increased (Mitsumoto, 1985). Statistical comparison between wobbler mice and controls [1] $P < 0.05$; [2] $P < 0.01$; [3] $P < 0.005$.

Lumbar ventral root myelinated nerve fibres

The total number of myelinated nerve fibres in the wobbler lumbar ventral roots was smaller than in controls at each age, but there was no significant difference between the two groups as a whole (Table 2). Although the number of large myelinated fibres diminished in wobbler mice as compared with controls ($P < 0.005$ at 6 weeks of age, $P < 0.01$ at 3 months of age), the number of small myelinated fibres increased ($P < 0.01$ at 6 weeks of age, $P < 0.05$ at 3 months of age), so that the total number of myelinated fibres was preserved. Despite such changes, a bimodal distribution was maintained (fig. 1).

The histograms in fig. 1 compare myelinated nerve fibres in the lumbar ventral roots with those in the cervical ventral roots obtained in a previous study (Mitsumoto and Bradley, 1982). The number of myelinated fibres of all sizes decreased in the cervical system at both 6 weeks and 3 months, so that wobbler mice failed to develop a bimodal distribution.

Axonal regeneration in hindlimb nerves

The distribution of transported radioisotopes distal to the crush site is shown for representative examples at 5 and 8 days in fig. 2. The pattern of axonal regeneration is nearly identical in both wobbler and control animals. Overall, there was no difference in outgrowth rate between these groups (Table 3, fig. 3). This differs from forelimb nerves of wobbler mice, which show slowing of the axonal outgrowth rate (Table 3).

DISCUSSION

In wobbler mice, the spared hindlimb motor system differed morphologically and functionally from the affected forelimb system. Although neurons undergoing vacuolar degeneration in the wobbler mouse are found diffusely throughout the central nervous

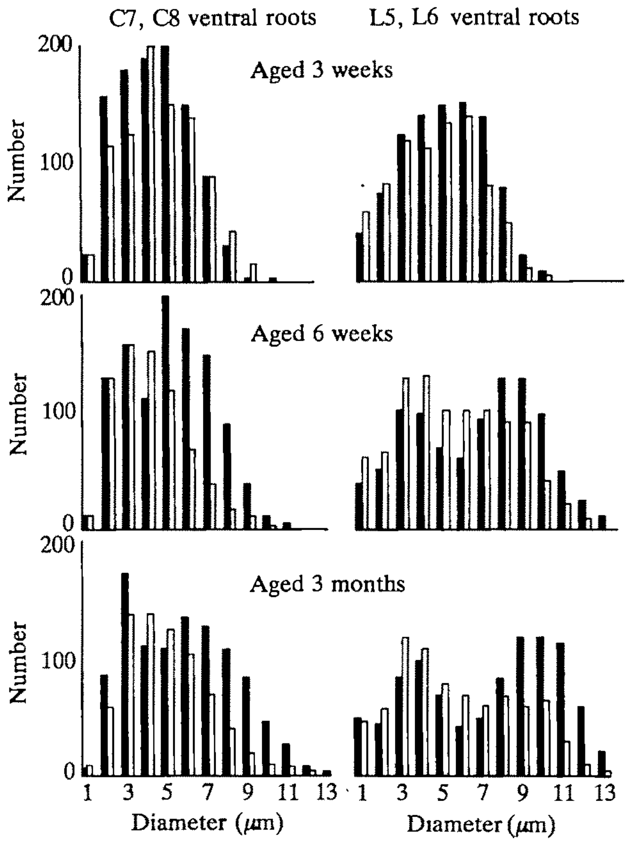

FIG 1. Size-frequency histograms for myelinated nerve fibres in lumbar ventral roots show a bimodal distribution at 6 wks and 3 mos of age both in wobbler and control animals, whereas the distribution remains unimodal in the wobbler cervical roots. In wobbler lumbar ventral roots, large myelinated fibres are diminished while small myelinated fibres (<6 μm) are increased. The histograms of cervical roots are shown for comparison (Mitsumoto and Bradley, 1982). In each group 4–6 animals were studied (*see* Table 2) Wobbler mice (☐), controls (■).

system, they are seen less frequently in the brain and lumbar anterior horns than in the cervical anterior horns, and rarely in the dorsal root ganglia (Duchen *et al.*, 1966, 1968; Mitsumoto and Bradley, 1985; Mitsumoto, 1988). Our histometric studies confirmed the previous observation that the lumbar region is less affected (Duchen *et al.*, 1968; Lewkowicz, 1979; Mitsumoto and Bradley, 1985). However, the vacuolated neurons in both regions are in a small minority. It is difficult to conceive that such minor differences in numbers of vacuolated neurons could produce the distinct clinical differences between the upper and lower limbs. Thus there can be little doubt that morphologically normal neurons produce the histometric changes of myelinated fibres and the functional disorder in this motor neuron disease (Mitsumoto, 1985; Mitsumoto and Gambetti, 1986; Mitsumoto and Boggs, 1987).

The histometric analysis of myelinated fibres showed that the lumbar system was less

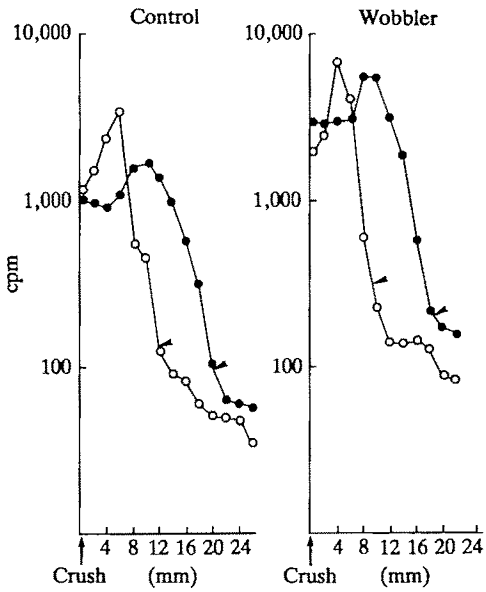

FIG. 2. Examples of axonal regeneration at 5 and 8 days after crush. The protein radioactivity conveyed by the fast component of axonal transport was measured in consecutive 2 mm nerve segments at 24 h after injection of isotope into motor nuclei. The peak radioactivity distal to the crush site represents a large cohort of regenerating axon tips. Arrowheads indicate the fronts of the fastest-growing axons, defined as having 10% of the protein radioactivity found in the first 2 mm nerve segment distal to the crush site (Mitsumoto, 1985). Five days (○), 8 days (●).

TABLE 3. DISTANCE OF AXONAL OUTGROWTH FROM CRUSH

Time	*Hindlimb (mean ± SEM)*		*Forelimb (mean ± SEM)**	
post-crush	*Controls*	*Wobbler*	*Controls*	*Wobbler*
5 days F[1]	11.3±0.4 mm (9)	12.1±1.1 mm (5)	8.9±0.4 mm (5)	8.3±0.3 mm (5)
8 days F	20.6±1.3 mm (9)	19.4±1.0 mm (9)	19.0±0.4 mm (6)	16.0±0.3 mm[2] (6)

[1] F = front of regeneration, i.e., the most distal location having 10% of the radioactivity at the crush site (*see* fig. 3). [2] The distance reached by the fastest growing axons in wobbler mice was significantly shorter than in controls ($P < 0.001$). * The results for the forelimb are from a previous study (Mitsumoto, 1985).

affected as the total number of myelinated fibres was preserved. This was because of an increased number of small myelinated fibres, in contrast to the cervical region where a loss of large fibres is seen without a compensatory increase in small fibres (Bradley and Jenkison, 1973; Lewkowicz, 1979; Mitsumoto and Bradley, 1982). A loss of large myelinated fibres is also seen in human motor neuron diseases (Chou and Nonaka, 1978; Robertson *et al.*, 1978; Kawamura *et al.*, 1981; Sobue *et al.*, 1981*a*, *b*; Bradley *et al.*, 1983). An increased number of small myelinated fibres, as found here in lumbar ventral

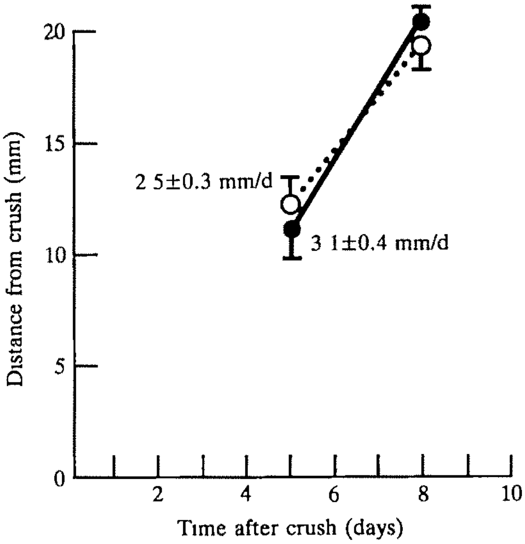

FIG. 3. Axonal outgrowth rates. The distance of the fastest-growing axons was the most distal location having 10% of radioactivity in the first 2 mm segment of nerve distal to the crush site (*see* Methods). At 5 and 8 days, the mean distances are plotted. The mean rate of regeneration is calculated by the least-squares methods. There was no significant difference in the rate of axonal elongation in either group. Wobbler, n = 14 (○), controls, n = 18 (●).

roots, has also been noted in the phrenic nerve of the wobbler mouse. The diaphragm continues to function and is spared from progressive denervation atrophy (Mitsumoto and Bradley, 1985). Thus, preservation of myelinated fibre numbers correlates with protection from progressive motor neuron disease.

There are three possible explanations for the compensation for a loss of large myelinated fibres by increased numbers of small myelinated fibres. First, the large fibres may not be depleted but may simply undergo atrophy, which would lead to an increased proportion of small myelinated fibres. This has been suggested to explain similar findings in the ventral roots and peripheral nerves in ALS (Inoue and Hirano, 1979; Bradley *et al.*, 1983). Histometric analysis of anterior horn cells in ALS in fact demonstrates neuronal atrophy (Tsukagoshi *et al.*, 1979; Kawamura *et al.*, 1981). Similarly, wobbler mice are found to have smaller anterior horn cells than normal controls (Murakami *et al.*, 1980). Although neuronal and axonal atrophy could explain the histometric pattern in the spared system it could only explain nerve fibre depletion in the affected system if it is concluded that severe neuronal atrophy leads to neuronal death. If this were the case, axonal atrophy might be expected in cervical roots at an early clinical stage (3 wks). However, there is no evidence for this in the findings shown in fig. 1, and pathological studies demonstrate no axonal degeneration (Mitsumoto and Bradley, 1982).

A second possibility is that lumbar ventral roots contain a significant number of regenerated axons, which are generally small. Because there is active axonal regeneration in the affected upper limb nerves (Mitsumoto and Bradley, 1982, 1985), a spared system might also contain regenerated nerve fibres. This might occur on a large enough scale to increase the number of small myelinated fibres and thereby compensate for the depletion of large fibres. However, the hindlimb nerves show no pathological evidence of axonal regeneration (Mitsumoto, 1988).

The third possibility is developmental delay. The histometric pattern in the wobbler hindlimb nerves never achieves that of normal adult animals (fig. 1) and there is no evidence of any regression in size of hindlimb axons. The disease begins at the time of rapid growth so that the growth of affected animals becomes impaired after 3 wks of age (Kozachuk *et al.*, 1987; Mitsumoto, 1988). The spinal cord, forelimb, and hindlimb nerves of wobbler mice are smaller and shorter than those of healthy controls (Bird *et al.*, 1971; Papapetropoulos and Bradley, 1972; Mitsumoto, 1988). The smaller size of the spinal cord and limb nerves appears to be part of a generalized developmental delay since all organs are proportionately small in wobbler mice. Growth retardation may thus influence the size of myelinated fibres in the unaffected hindlimb nerves and contribute to the similar histometric change in the phrenic nerve (Mitsumoto and Bradley, 1985).

We found normal axonal regeneration in the less affected hindlimb nerves, in clear contrast to the forelimb (Mitsumoto, 1985). This suggests that these motor neurons possess ‘disease resistance’ (Mulder and Howard, 1976; Mitsumoto *et al.*, 1988). In the cervical motor neurons, spontaneous axonal regeneration is seen (Mitsumoto and Bradley, 1982), associated with increased collateral sprouting and muscle fibre type grouping (Duchen *et al.*, 1966, 1968; Mitsumoto, 1988). While this suggests that their regenerative process may be enhanced, regenerative capacity after axotomy is actually reduced (Mitsumoto, 1985). Axonal regeneration is determined by two intrinsic factors of the neuron: (1) its ability to manufacture macromolecules required for axonal elongation (Lasek, 1970; Grafstein and McQuarrie, 1978; McQuarrie and Grafstein, 1982), and (2) its ability to transport these materials to regenerating axon tips (McQuarrie, 1983). By synthesizing these building blocks, the nerve cell body plays a major role in axonal regeneration (Grafstein and McQuarrie, 1978). Neuronal RNA metabolism, protein synthesis and axonal transport are decreased in wobbler cervical anterior horn cells (Bird *et al.*, 1971; Murakami *et al.*, 1980, 1981; Kurahashi *et al.*, 1986; Mitsumoto and Gambetti, 1986; Mitsumoto *et al.*, 1990), which probably accounts for impaired regeneration after axotomy (Mitsumoto, 1985). In contrast, the normal regeneration seen after axotomy of the lumbar anterior horn cells suggests that RNA metabolism, protein synthesis, and axonal transport are normal.

One possible explanation why a certain group of anterior horn cells is more affected than others in this hereditary disease is that a defective gene may be more frequently ‘switched on’ in one group of cells than in others. This, perhaps, is related to ontogenetic or developmental differences among the groups of motor neurons (Lemire *et al.*, 1975). A gene product derived from the gene in question may be required more in one group of motor neurons than in others. Thus a phenotypic defect may become more pronounced in these motor neurons. If this is the case, there might be a genetic specificity for certain groups of anterior horn cells leading to disease resistance or disease vulnerability.

However, it is also possible that a genetically determined feedback mechanism from the periphery (such as from muscle) may be altered, so that either positive feedback is reduced or inhibitory feedback is enhanced (Appel, 1981). Although our studies do not yet explain why selective neuronal involvement occurs in wobbler mouse motor neuron disease, the histometric, regenerative, and functional differences between the affected and spared systems provide important clues for further analysis.

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CHOREIC MOVEMENTS IN THE MACAQUE MONKEY INDUCED BY KAINIC ACID LESIONS OF THE STRIATUM COMBINED WITH L-DOPA

PHARMACOLOGICAL, BIOCHEMICAL AND PHYSIOLOGICAL STUDIES ON NEURAL MECHANISMS

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SUMMARY

In order to produce choreic movements in monkeys (*Macaca* species), we have used the combination of unilateral kainic acid injections into the striatum and the systemic administration of L-DOPA. Two control monkeys received kainic acid injections alone and 7 were also given L-DOPA (150–300 mg/day) orally. No spontaneous involuntary movements were seen after kainic acid injection alone (over a period of 4–14 days). In the 7 monkeys that received L-DOPA 4–7 days after the kainic acid injection, in 5, whose striatal lesions were located in the rostral dorsolateral striatum, choreic movements appeared in the contralateral limbs following each dose of L-DOPA, whereas in 2 monkeys in which the lesion was large enough to occupy more than 60% of the rostral striatum, no choreic movements occurred. Preservation of some part of the striatum is therefore necessary for the generation of choreic movements. A considerable number of neurons in the rostral ventromedial putamen, which escaped the kainic acid lesion showed burst discharges when L-DOPA was administered. These were not seen in the caudate nucleus. The burst discharges recorded from some neurons were time-locked to the choreic movements. Since deafferentation by dorsal root section had no effect, these burst discharges appeared to be generated in the unlesioned striatum during the choreic movements. Brains of 6 monkeys were analysed biochemically for γ-aminobutyric acid concentration, choline acetyltransferase and tyrosine hydroxylase activity, and spiroperidol binding. Concentrations of γ-aminobutyric acid, choline acetyltransferase activity and spiroperidol binding in the basal ganglia did not correlate with the generation of choreic movements. A finding which appeared to have a clear correlation was an increase in excess of 50% in tyrosine hydroxylase activity in the rostral ventromedial striatum on the lesioned side which escaped the kainic acid lesion in choreic monkeys as compared with the corresponding area on the intact side. It is, therefore, assumed that certain populations of 'activated' nigrostriatal dopaminergic neurons which innervate the unlesioned area of the striatum may be more likely to attain a level of activity, induced by L-DOPA, sufficient to produce choreic movements in the contralateral limbs. Methamphetamine, but not apomorphine, given to 2 monkeys, produced an effect that was essentially the same as that of L-DOPA. These results support the hypothesis that the 'activated' dopaminergic components in choreic monkeys are the presynaptic dopaminergic nerve terminals rather than the postsynaptic dopaminergic receptors.

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INTRODUCTION

Huntington's disease is a progressive inherited degenerative disease of the central nervous system characterized clinically by choreic movements and cognitive deterioration. Although the genetic defect has recently been assigned to the abnormal DNA sequence(s) localized at the tip of the short arm of chromosome 4 (Gilliam *et al.*, 1987), many questions remain unanswered. Particularly important, from a clinical standpoint is the lack of understanding of the mechanisms underlying the generation of choreic movements. The neuropathology of Huntington's disease suggests that the choreic movements are closely correlated with a loss of neurons from the striatum, both from the caudate nucleus and the putamen (Dunlap, 1927). Extensive modern analyses of neurotransmitter markers in the basal ganglia of patients dying from Huntington's disease have revealed that concentrations of γ-aminobutyric acid (GABA) and its synthesizing enzyme (GAD) (Perry *et al.*, 1973; Bird and Iversen, 1974), concentrations of substance P (Kanazawa *et al.*, 1977*b*) and methionine-enkephalin (Emson *et al.*, 1980) in the globus pallidus and/or the substantia nigra are all markedly decreased. The above substances are markers for small striatal efferent neurons which send long axons to the globus pallidus and/or the substantia nigra (Yoshida *et al.*, 1972; Kanazawa *et al.*, 1977*a*; Hong *et al.*, 1977). Indeed, a clear, positive correlation between the degree of a loss of small striatal neurons and the decreases in GABA and substance P in the globus pallidus and/or the substantia nigra has now been confirmed (Kanazawa *et al.*, 1985). Nevertheless, it is difficult to attribute the generation of choreic movements to the decrease of these neurotransmitters for the following reasons: (1) attempts to raise GABAergic function in Huntington's disease brain have failed to have any beneficial effects on choreic movements (Shoulson, 1979); (2) concentrations of substance P in the basal ganglia do not correlate well with the severity of choreic movements of Huntington's disease (Kanazawa *et al.*, 1985); and (3) it is extremely rare to find choreic movements in Pick's disease, even in cases where the striatum exhibits a severe atrophy with a corresponding loss of GABA and substance P from the basal ganglia, as in Huntington's disease (Kanazawa *et al.*, 1988). In addition to those neurotransmitters belonging to the striatal efferent neurons, a marker of cholinergic interneurons of the striatum, choline acetyltransferase (ChAT), is also decreased in the striatum in Huntington's disease, corresponding to the degeneration of large neurons in the striatum (Bird and Iversen, 1974; Kanazawa *et al.*, 1988). Since anticholinesterase drugs have little beneficial effect on choreic movements, the decrease of ChAT activity cannot be regarded simply as the causative biochemical change in the generation of choreic movements.

Another possibly important biochemical finding in Huntington's disease is the normal or enhanced levels of the dopamine and catecholamine synthesizing enzyme, tyrosine hydroxylase (T-OH) activity in the striatum and the substantia nigra (Bird and Iversen, 1974; Bird *et al.*, 1980). This indicates that the presynaptic components of the nigrostriatal dopaminergic neurons remain intact. Indeed, choreic movements in Huntington's disease are readily supressed by the administration of antidopaminergic neuroleptics. Moreover, some of the subjects at risk for Huntington's disease manifest choreic movements following challenge with the dopamine precursor, L-DOPA (Klawans *et al.*, 1980).

We have sought to develop a primate model of chorea, based on the neuropathological features of Huntington's disease. Several previous studies have sought to produce involuntary movements in monkeys by making electrolytic lesions in the striatum (Richter and Hines, 1938; Mettler and Mettler, 1942; Kennard, 1944; Davis, 1958). However, none was able to simulate 'choreic movements' in monkeys. In fact, electrolytic or electrothermal lesions destroy not only striatal neurons but also afferent and fibres en passage. This may be one of the reasons why experimental choreic movements cannot be produced by such lesions, even in monkeys given an L-DOPA challenge (Sax *et al.*, 1973). It is well known that the administration of L-DOPA can cause dyskinetic choreic involuntary movements in monkeys whose nigrostriatal dopaminergic system has undergone degeneration accompanied by a secondary dopamine supersensitivity in the striatum (Lee *et al.*, 1978).

As an alternative type of model, striatal lesions produced by a excitotoxic substance such as kainic acid or quinolinic acid are more similar to the pathology of Huntington's disease than electrolytic lesions, because afferent and en passage fibres are spared (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). Although these substances can simulate the biochemical and pathological features of the basal ganglia in Huntington's disease, involuntary movements similar to choreic movements cannot be reproduced simply by the striatal injection of excitotoxins either in rodents (Coyle *et al.*, 1978) or in monkeys (Kanazawa *et al.*, 1986). A third type of model in monkeys is that produced transiently by the local injection of the GABA antagonists picrotoxin or bicuculline into the striatum (Crossman *et al.*, 1984, 1987). Involuntary movements evoked by these drugs are choreic, ballistic, dystonic or myoclonic. The latter two are rarely encountered in Huntington's disease. We have attempted to produce choreic movements in monkeys (Kanazawa *et al.*, 1986) by the combined use of striatal kainic acid injections and the systemic administration of L-DOPA. The aim of the present study was therefore to validate this method for producing choreic movements in monkeys and to investigate the underlying biochemical, pharmacological and physiological alterations in the basal ganglia of the treated monkeys.

METHODS

Experiments were performed on 9 young adult male monkeys (body wt 3.6–4.3 kg) of 6 laboratory-bred *Macaca fascicularis*; 2, as controls, received a striatal kainic acid injection alone (C1 and C2); 4 received a kainic acid injection and L-DOPA administration, 1 being used for behavioural and morphological studies (M6), 1 for behavioural, morphological and pharmacological studies (M7) and 2 for behavioural, morphological and biochemical studies (M1, M2). Two *Macaca fuscata* (M3 and M4) received a kainic acid injection and L-DOPA administration and were used for behavioural, morphological, biochemical and physiological studies and 1 *Macaca fuscata* (M5) received a kainic acid injection and L-DOPA, and apomorphine or metamphetamine administration for behavioural studies All surgical procedures were performed under general anaesthesia with ketamine (15 mg/kg). The experiments were performed in accordance with the rules for animal care and management of the Tsukuba Primate Centre as well as with the guidance principles for experiments on nonhuman primates formulated by the Primate Society of Japan as formulated in 1986.

Kainic acid injection into the striatum

Under general anaesthesia, a gas chromatography needle (0 3 mm outer diameter) was inserted unilaterally under stereotaxic control into the dorsolateral part of the striatum on the right. The positions of the needle tip for the injection of kainic acid into the caudate nucleus in monkeys C1, C2, M2, M3, M4 and M5 were (1) AP:22.0 mm, L:6.0 mm, H:24.5 mm; (2) AP:19.0 mm, L:6.0 mm, H:25.0 mm; (3) AP:16.0 mm, L·6.0 mm, H:25.5 mm. The positions of the needle tip for the putamen in these monkeys were (1) AP:22.0 mm, L:10.5 mm, H:20.5 mm; (2) AP:19.0 mm, L:11.0 mm, H:21.0 mm; (3) AP:16.0 mm, L:12.5 mm, H:21.0 mm. In M1, additional injections were performed to the ventromedial striatum as follows: (1) AP:22.0 mm, L:4.0 mm, H:22.5 mm; (2) AP:19.0 mm, L:4.0 mm, H:23.0 mm, for the caudate nucleus; and (3) AP:22.0 mm, L:8.5 mm, H:18.0 mm, (4) AP:19.0 mm, L:9.0 mm, H.18.0 mm, for the putamen.

Kainic acid (Sigma) was dissolved in physiological saline at a concentration of 3.0 mg/ml; 1 μl (containing 3.0 μg = ~14 pmol kainic acid) of this solution was injected into each of the above positions at a rate of 1.0 μl/2 min. After the injection, the needle was left in place for at least 2 min in order for possible high pressures at the injection site to subside.

Schedule of L-DOPA administration

As shown in Table 1, C1 and C2 did not receive any drugs until they were killed On the fourth postoperative day, monkeys M1, M2 and M5 started to receive L-DOPA with a DOPA decarboxylase inhibitor (100 mg L-DOPA+20 mg benserazide/tablet) orally once a day each morning, whereas in M3 and M4 this was commenced on the seventh postoperative day. M1, M2, M6 and M7 received 300 mg of L-DOPA with 60 mg of benserazide in the form of tablets for 5 days. In M3, M4 and M5, the tablets were crushed and suspended in orange juice; the L-DOPA (+benserazide) was initially 300 mg/day and was then reduced to 150 mg/day as shown in Table 1.

Schedule of pharmacological studies

As shown in Table 1, on the sixth day after the final dose of L-DOPA, M5 and M7 received a subcutaneous injection of apomorphine solution (1 mg/ml) at a dose of 0.3 mg/kg and their motor behaviour was observed for 1 h. On the ninth day after the final dose of L-DOPA, M5 and M7 received methamphetamine (3 mg/ml) subcutaneously in a dose of 2 mg/kg and observed for 1 h

Behavioural observations

After the kainic acid injection, the monkeys were kept in separate cages, 45 cm×60 cm×60 cm in size. The behaviour of C1, C2, M1, M2, M6 and M7 was recorded by a videotape recorder placed in front of the cage for several hours after the L-DOPA administration and analysed subsequently. The heads of M3, M4 and M5 were restrained in the monkey chairs just before the L-DOPA administration. The behaviour of these animals was also recorded on a videotape.

Electrophysiological investigation of striatal neurons

The monkey's head was restrained during the recording of neuronal activity which employed the methods of Evarts (1966, 1968). Single neuron activity was recorded by introducing glass-coated Elgiloy microelectrode (Suzuki and Azuma, 1976) through an implanted recording chamber, with a conventional amplifier and filter system. The recorded neuronal activity was displayed on a cathode ray oscilloscope. The onset time of discriminated spikes of single neurons was fed to a computer (PDP 11/23) through a digital input device. Interspike interval and mean frequency of impulse discharges were measured by the computer.

EMG recordings were obtained from arm muscles (biceps and triceps brachii) throughout the single

TABLE 1. TIME SCHEDULE OF EXPERIMENTS*

Day	*C1*	*C2*	*M1*	*M2*	*M3*	*M4*	*M5*
0	Kainic acid injection into right striatum						
1							
2							
3							
4			300	300			300
5			300	300			300
6			300	300			250
7			300	300	300	300	150
8			300	300	300	200	
9					200	150	
10					150	RS	
11							
12							
13						150	Apo
14	†	†	†	†		150	
15						150	
16							Met
17							†
18					†		
19						†	

* The values 300, 250, 200 and 150 represent doses (in mg) of L-DOPA administered orally, 300 = 3 tablets of L-DOPA+benserazide (*see* text for details) † = killed, RS = cervical dorsal root section, Apo = apomorphine injection, Met = methamphetamine injection.

unit recording sessions. Microelectrode penetrations were localized on histological sections of the brain by comparing the pattern of electrode tracks and microlesions with descriptions of the penetrations during recording session.

Unilateral dorsal root section

On the day following the final dose of L-DOPA, M4 was anaesthetized again with pentobarbitone (15 mg/kg). A skin incision was made in the midline of the dorsal neck. After laminectomy, the tip of a ball electrode was attached on the surface of the left dorsal column. On stimulation of the left ulnar nerve, a field potential was evoked at the ball electrode. The dorsal roots on the left from C4 to T2 were then extensively sectioned with small scissors. The success of the section was tested by the disappearance of the evoked field potential elicited by ulnar nerve stimulation.

Methods for evaluation of kainic acid lesions and to dissect brain areas for sampling

All monkeys were deeply anaesthetized with pentobarbitone (50 mg/kg) on the day indicated in Table 1, 4−8 days after the last dose of L-DOPA, and killed by decapitation. The brain was quickly removed from the skull and cut into 5 mm slices in the frontal plane. These were frozen on dry ice as rapidly as possible as described previously (Kanazawa, 1983).

The slices were cut into serial 200 μm frozen sections with a cryostat microtome at −15° C, during which photographs were taken at approximate intervals of 1.2 mm. The extent of the kainic acid lesions was clearly recognizable (fig. 1). Using a computer-assisted 'area' calculator (Kontron Videoplan), the approximate relative size (volume) of lesions against the total size (volume) of the striatum was calculated from these photographs (Table 2).

Frozen sections were attached to Perspex plates, and several regions in the basal ganglia were dissected in a cold box (inner temperature −15° C to −20° C) under a binocular microscope using a small dissection knife. The *rostral striatum* of the lesioned side was divided into 3 parts (fig. 2): (1) dorsolateral caudate nucleus and (2) dorsolateral putamen, both of which were lesioned by kainic acid, and (3) the remaining ventromedial striatal area which escaped the kainic acid lesion. The *caudal striatum* of the lesioned side was dissected as shown in fig. 2. The intact side of the striatum was divided in exactly the same manner as the lesioned side. The inner and the outer segments of the globus pallidus were dissected separately A tissue sample of substantia nigra was taken from the rostral and the caudal sections.

Four consecutive sections were used for determination of neurotransmitter markers as follows: (1) amino acids (GABA, glutamate and aspartate), (2) choline acetyltransferase (ChAT); (3) tyrosine hydroxylase (T-OH); and (4) spiroperidol binding

Methods for biochemical analysis

GABA concentrations were determined by the enzymatic and fluorometric method of Graham and Aprison (1966) GABAase was purchased form Boehringer Mannheim ChAT activity was determined by the

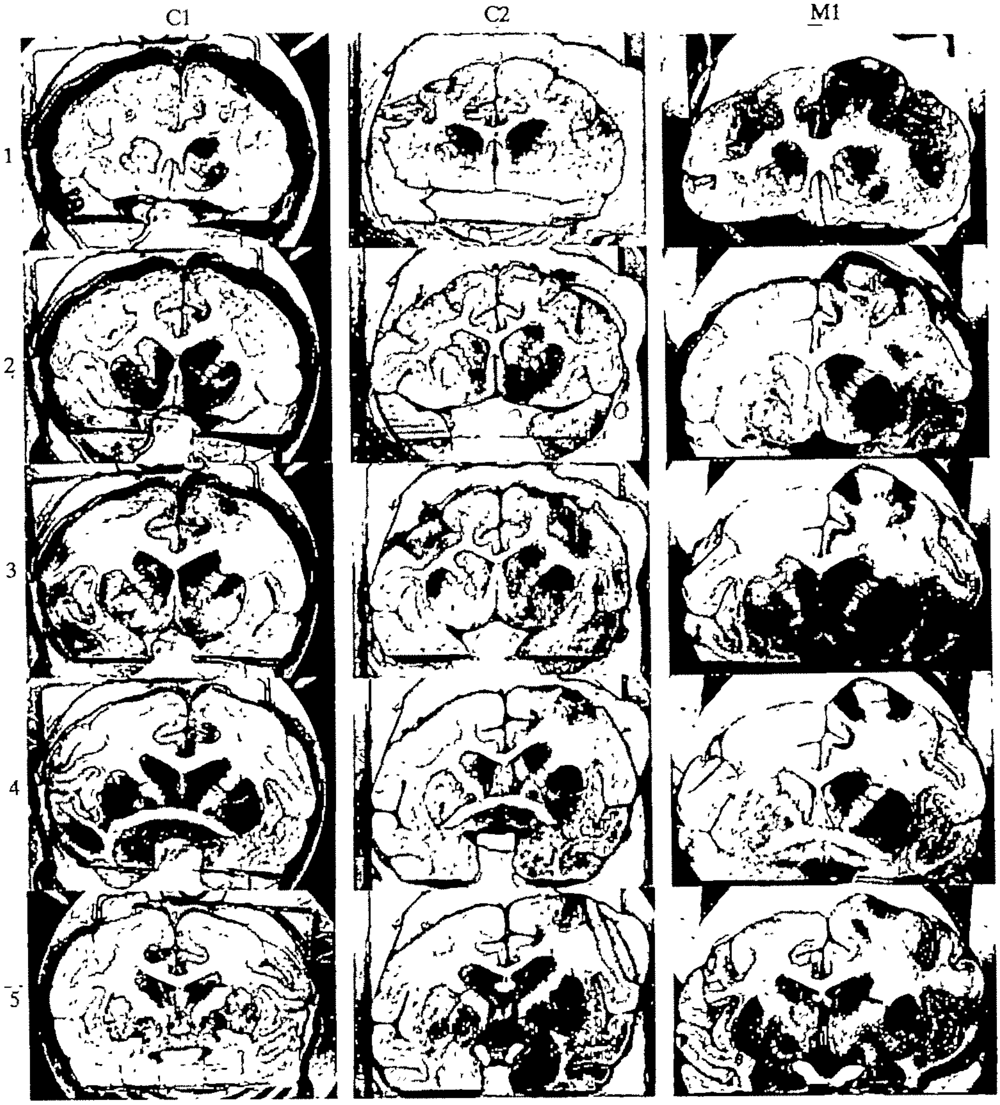

FIG 1

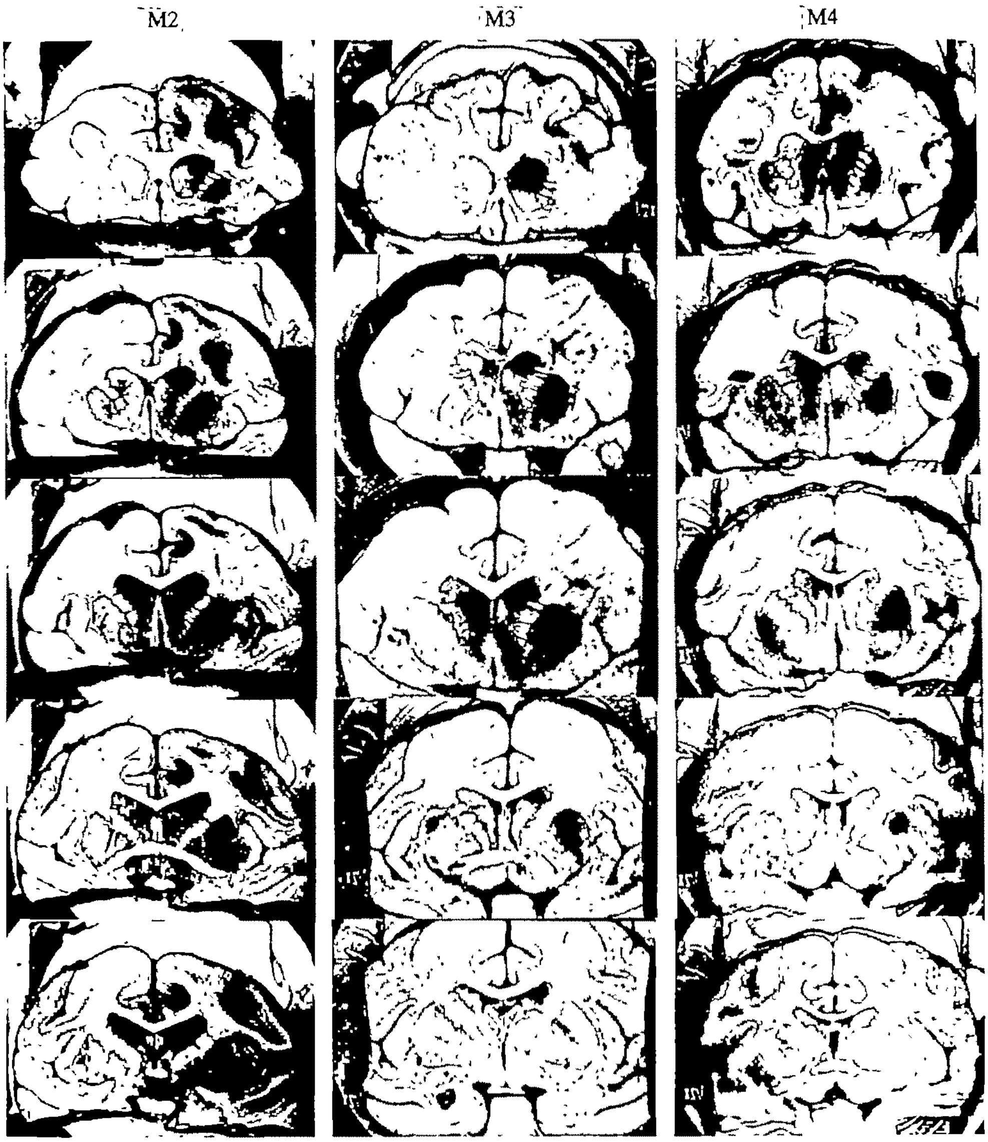

FIG 1. Photographs of frozen monkey brains showing the extent of kainic acid lesions which are recognizable by their dark colour. Photographs are numbered from rostral to caudal The lesions in the right hemispheres were well localized and involved the caudate nucleus, the putamen and part of the cerebral cortex. The approximate relative size of lesions against the total size of the striatum was calculated from these photographs (*See* also facing page)

radiochemical method of Fonnum (1969), using [^{3}H]acetyl-CoA (Amersham). T-OH activity was determined by the radiochemical method of Hendry and Iversen (1971), using [^{3}H]tyrosine. Spiroperidol binding was assayed by Dr Norio Ogawa, Okayama University, by the method of List and Seeman (1981), using spiroperidol as a ligand. B_{max} was read on the Scatchard plot obtained from at least 10 different concentrations of ligand. Protein content was determined by the method of Lowry *et al.* (1951)

TABLE 2 APPROXIMATE PERCENTAGE VOLUME OF THE STRIATAL LESIONS PRODUCED BY KAINIC ACID*

Monkey	C1	C2	M1	M2	M3	M4	M6	M7
Caudate nucleus								
Rostral	56	23	62	45	49	34	48	47
Caudal	68	52	64	57	46	0	17	25
Putamen								
Rostral	26	18	65	37	59	49	84	56
Caudal	0	50	62	46	27	14	33	30

* Values of 'rostral' were calculated from photographs 1, 2, 3, in fig 1 and those of 'caudal' from photographs 4, 5, in fig 1

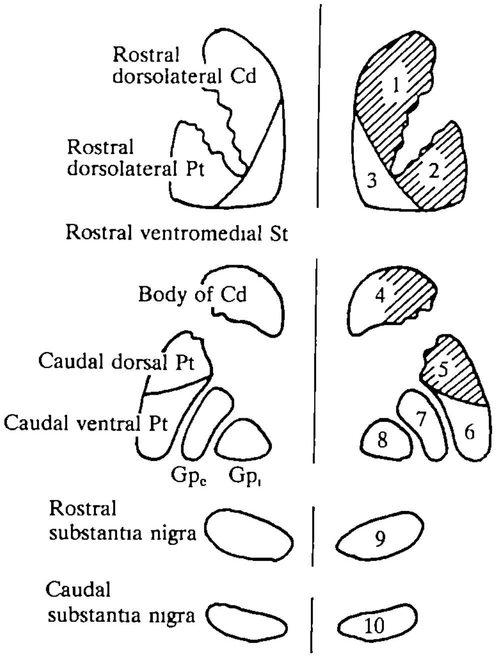

Fig. 2 Schematic drawings of the method of tissue dissection for sampling Shaded areas represent kainic acid lesions The numbers 1–10 correspond to those in Tables 3–6. Cd = caudate nucleus, Pt = putamen, Gp_e and Gp_i = external and internal segments to globus pallidus

RESULTS

Size and location of the striatal kainic acid lesions

Although the positions of the injection needle for kainic acid were approximately the same, the size and the location of lesions varied, probably due to difference in size of brain. Fig. 1 illustrates the actual lesion of each monkey with photographs taken at about 1.2 mm intervals. In addition, the approximate size of the lesion was calculated from these photographs and expressed as a percentage volume of the striatum (Table 2). Histological observation revealed that the extent of the discolouration was exactly the same as the extent of neuronal damage. Based on these results, the monkeys were divided into three groups as follows: (1) striatal lesions that were mainly located rostral to the anterior commissure (25–60% of the rostral striatum destroyed) (C1, M2, M3, M4, M7); (2) lesions that were mainly located around the anterior commissure (less than 25% destruction of the rostral striatum) (C2); (3) striatal lesions that were large enough to include both rostral and caudal striatum (more than 60% of the rostral striatum destroyed) (M1, M6). In every instance except M1 and M6, lesions were located in the dorsolateral part of the caudate nucleus and the putamen. In these 2 monkeys, the lesions were not confined to the dorsolateral striatum but extended to the ventromedial part of the striatum.

Spontaneous behaviour of monkeys after the unilateral striatal kainic acid injection alone

All monkeys started to show myoclonic or seizure-like (not choreic) involuntary movements of the contralateral limbs several hours after the kainic acid injection. These spontaneous movements subsided within several hours or a half day. Except for this, we did not see any spontaneous involuntary movements over 4–7 days after the striatal kainic acid injection, that is, before the L-DOPA administration. In C1 and C2, which did not receive L-DOPA, no choreic movements during the period up until they were killed on the fourteenth day after the kainic acid injection. Poverty of voluntary movements of the contralateral limbs was noted in all monkeys of varying severity, most severe in M1 and least in C2. In all animals, however, the apparent 'paresis' disappeared within a few days of the kainic acid injection.

Involuntary choreic movements of the contralateral limbs induced by L-DOPA

When L-DOPA plus a DOPA decarboxylase inhibitor was administered, M1 and M6 showed only a form of dystonic posturing in the left (contralateral) limbs, and no choreic movement appeared. On the other hand, M2, M3, M4, M5 and M7 started to exhibit clearly recognizable 'choreic' and sometimes 'ballistic' movements affecting exclusively the contralateral limbs (predominantly the upper limb in all cases), and oral dyskinesias lasting for 5–6 h after the second or third dose of L-DOPA. The same responses were obtained subsequently if the same dose of L-DOPA was administered. When the dose of L-DOPA was reduced from 300 mg to 200 mg or 150 mg, the appearance of the involuntary movements was delayed and its magnitude was less severe than following 300 mg.

The time between L-DOPA administration and the appearance of choreic movements differed from animal to animal, but the interval in M2 and M7, when L-DOPA was given as tablets, was much longer (2 h) than in M3, M4 and M5 when it was given as a suspension in orange juice (30 min–1.5 h, depending on the dose).

The involuntary movements were characterized by a rather stereotyped abrupt series of limb movements on the left side, contralateral to the kainic acid injection. For instance, one series of movements began with adduction of the upper arm and proceeded to flexion and extension of the forearm, then further adduction of the upper arm, flexion of the hand, followed by flexion and extension of the forearm again, and finally abduction of the upper arm. The latter part of this series of movement had a flinging quality. These abnormal movements were also observed in the left hindlimb but sometimes less vigorously than those in the upper limb. Occasionally the series of involuntary movements continued without long interruptions, similar to ballism. Less frequently, twisting movements of the forearm or hindlimb were observed. The oral dyskinesias that were observed were not lateralized.

Although, in each animal, the first response that appeared after the administration of L-DOPA was the occurrence of the above-mentioned choreic movements in the contralateral upper limb, generalized stereotyped hyperactive behaviour also appeared bilaterally. These generalized DOPA-induced effects began 30 min to 2 h after the onset of the left-sided choreic movements and coexisted with them afterwards (fig. 3A). Choreic movements were easily distinguished from hyperkinetic movements because of their aimless character.

Involuntary movements induced by the administration of apomorphine or methamphetamine

The effects of the dopamine agonists apomorphine and methamphetamine were examined in M5 and M7. As shown in fig. 3A, M5 started to exhibit choreic movements of the left upper limb 40 min after the oral administration of 150 mg of L-DOPA. These effects subsided after 6 h.

When M5 received a 1.2 mg (0.3 mg/kg) subcutaneous injection of apomorphine (fig. 3B), he began to show generalized hyperkinesia 5 min after the injection, lasting 30 min, without developing clear choreic movements confined to the left limbs. On the other hand, when M5 received an 8 mg (2 mg/kg) subcutaneous injection of methamphetamine (fig. 3C), he started to exhibit definite choreic movements of the left upper limb 5 min after the injection. As for L-DOPA, a phase of generalized hyperkinesia was then superimposed on the left-sided choreic movements. These phenomena were also observed in M7.

Alterations of neurotransmitter markers in the basal ganglia of monkeys

Gamma-aminobutyric acid (GABA) concentration. GABA concentrations in the various areas in the basal ganglia of 6 monkeys are listed in Table 3. There was a difference in GABA levels in basal ganglia areas on the intact side between L-DOPA treated monkeys (M1, 2, 3, 4) and untreated monkeys (C1 and C2). The magnitude of difference was rather constant, being 1.3 – 1.5 times higher in untreated monkeys.

GABA levels in the rostral lesioned areas, i.e., the dorsolateral caudate nucleus and the putamen, were markedly decreased compared with those of the corresponding areas of the intact side. However, it is noteworthy that the GABA concentrations in the caudal dorsal area of the putamen, which was involved in the kainic acid lesion, were not

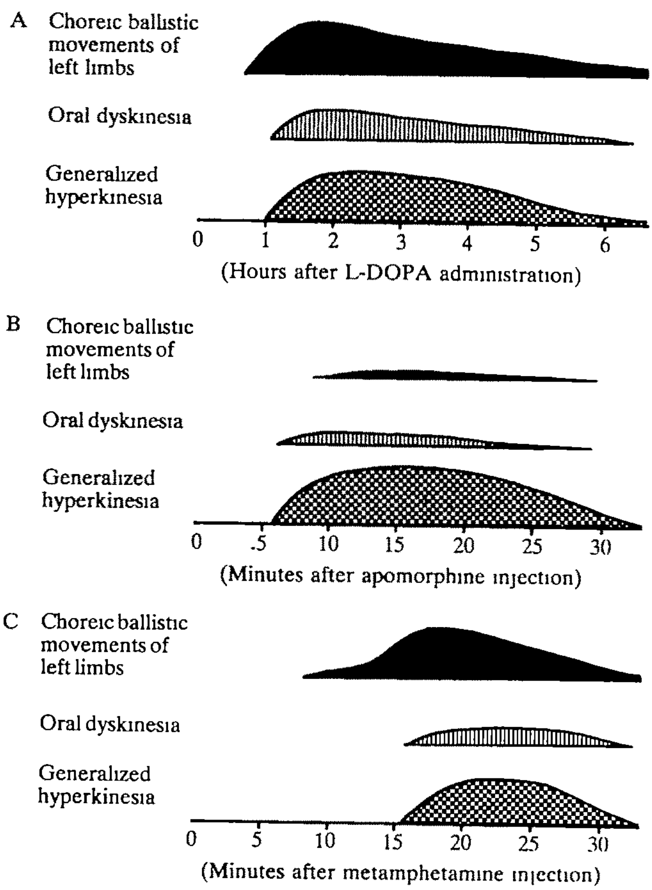

FIG 3 Time course of the effects of L-DOPA (A), apomorphine (B) and methamphetamine (C) on motor behaviour in monkey M5. L-DOPA was administered 7 days after the kainic acid injection at a dose of 150 mg with 30 mg of benserazide. Apomorphine and methamphetamine were injected subcutaneously 13 and 16 days after the kainic acid injection, in doses of 1.2 mg and 8 0 mg, respectively.

decreased in C1 and M3. There was no significant change ('significant' means an increase or decrease by more than one-third of the original value, i.e., 33.3%) of GABA levels in the rostral ventromedial striatum which escaped the kainic acid lesion. A large decrease of GABA occurred in the substantia nigra and the globus pallidus in M1, where destruction of the striatum was most extensive. Conversely, only a slight decrease in GABA in these nuclei was found in C2, whose striatal lesions were the smallest.

Choline acetyltransferase (ChAT) activity. ChAT activities in the areas of the basal ganglia of each monkey are shown in Table 4. Activities in the rostral lesioned areas, i.e., the dorsolateral caudate nucleus and the putamen, were greatly decreased as compared with those of the corresponding areas of the intact side. Changes in ChAT activities in the caudal striatum varied from animal to animal. There was no significant change of ChAT activities in the rostral ventromedial striatum. There was no significant

TABLE 3 GAMMA-AMINOBUTYRIC ACID (GABA) (μmol/g TISSUE)

Monkey		*C1*		*C2*		*M1*		*M2*		*M3*		*M4*	
		Intact side	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*
Rostral striatum													
Dorsolateral caudate	1	4.0	0.7 (−82%)	4.8	1.2 (−75%)	3.9	0.5 (−87%)	2.7	0.6 (−78%)	2.5	0.6 (−75%)	3.1	0.3 (−90%)
Dorsolateral putamen	2	5.0	1.5 (−70%)	4.3	1.2 (−72%)	3.7	0.4 (−89%)	2.5	0.6 (−76%)	2.8	0.6 (−77%)	4.2	0.3 (−93%)
Ventromedial striatum (incl. nucleus accumbens)	3	7.2	6.0	5.2	4.7	4.6	4.2	3.3	3.1	3.0	2.1	4.4	3.8
Caudal striatum													
Body of caudate	4	4.0	4.3 (+8%)	3.5	2.0 (−43%)	3.4	4.0 (+18%)	2.4	1.4 (−42%)	3.1	0.9 (−73%)	2.9	3.1 (+7%)
Dorsal putamen	5	4.6	4.5 (−2%)	3.8	1.3 (−66%)	3.9	0.8 (−79%)	2.4	1.4 (−42%)	2.3	2.3 (−1%)	4.1	1.3 (−68%)
Ventral putamen	6	5.0	6.7 (+34%)	4.6	4.3 (−7%)	3.9	3.9 (0%)	3.6	3.1 (−14%)	3.3	2.6 (−22%)	4.2	4.0 (−5%)
Globus pallidus													
Ext segment	7	14.8	10.3 (−30%)	13.5	10.8 (−20%)	10.9	3.5 (−68%)	12.9	10.4 (−19%)	8.8	8.4 (−4%)	10.3	6.2 (−40%)
Int segment	8	14.6	5.6 (−60%)	16.2	13.8 (−15%)	6.7	1.8 (−73%)	14.9	9.8 (−34%)	11.1	3.1 (−72%)	11.3	7.2 (−36%)
Substantia nigra													
Rostral part	9	13.9	10.4 (−25%)	15.2	11.9 (−22%)	11.3	6.3 (−44%)	12.7	10.1 (−20%)	11.3	6.2 (−45%)	9.7	6.4 (−34%)
Caudal part	10	10.1	7.2 (−29%)	12.2	10.7 (−12%)	7.7	2.9 (−62%)	11.8	11.7 (−1%)	7.5	6.3 (−16%)	9.1	6.8 (−25%)

TABLE 4 CHOLINE ACETYLTRANSFERASE (ChAT) (nmol/mg PROTEIN/h)

Monkey		*C1*		*C2*		*M1*		*M2*		*M3*		*M4*	
		Intact side	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*
Rostral striatum													
Dorsolateral caudate	1	53.0	18.8 (−65%)	49.9	14.0 (−72%)	56.2	39.1 (−30%)	52.2	23.3 (−55%)	65.5	26.9 (−59%)	45.9	2.6 (−94%)
Dorsolateral putamen	2	63.0	32.9 (−48%)	59.4	31.4 (−47%)	73.4	29.5 (−60%)	78.1	39.3 (−50%)	40.8	26.1 (−36%)	66.5	26.5 (−60%)
Ventromedial striatum (incl. nucleus accumbens)	3	67.4	70.4 (+4%)	52.9	55.5 (+5%)	67.6	56.0 (−17%)	73.1	53.3 (−27%)	74.9	69.2 (−8%)	50.5	51.6 (+2%)
Caudal striatum													
Body of caudate	4	63.7	70.8 (+11%)	67.2	20.9 (−69%)	73.1	74.2 (+2%)	78.0	43.9 (−44%)	77.2	24.0 (−69%)	70.1	56.9 (+19%)
Dorsal putamen	5	85.6	85.4 (0%)	81.6	36.6 (−55%)	80.2	39.6 (−51%)	82.0	53.3 (−45%)	67.6	78.9 (−17%)	57.8	58.4 (+1%)
Ventral putamen	6	108.9	100.5 (−8%)	82.7	75.1 (−9%)	80.0	68.1 (−15%)	76.4	91.6 (+20%)	60.8	80.7 (+33%)	46.3	50.0 (+8%)

difference in ChAT activities in the intact side between DOPA-treated and untreated monkeys.

Tyrosine hydroxylase (T-OH) activity. Activities in basal ganglia are listed in Table 5. Both in the rostral and caudal striatum of the intact side they were significantly higher in DOPA-treated than in untreated monkeys. T-OH activities in the substantia nigra of the intact side also showed the same tendency, but the differences were smaller.

Changes in T-OH activity in the striatal lesioned areas varied from area to area when compared with the intact side. It is noteworthy, however, that activities in the ventromedial striatum on the lesioned side, which escaped the kainic acid, were much higher than those in the corresponding area on the intact side in C1, M2, M3 and M4, whose rostral striata were destroyed by approximately 25−60% (Table 2). On the other hand, in C2 and M1, where the volumes of rostral striatal lesions were less than 25% or more than 60%, respectively, no such relative increase in activity was found in the ventromedial striatal areas which escaped the kainic acid lesion.

In C1 and C2, not treated with L-DOPA, T-OH activities in the substantia nigra on the lesioned side were significantly decreased. On the other hand, in DOPA-treated monkeys, with the exception of M2, T-OH activities in the substantia nigra on the lesioned side were increased as compared with those on the intact side.

Spiroperidol binding activity. Binding values in the rostral striatal areas are summarized in Table 6. Spiroperidol binding on the intact side of monkeys untreated with L-DOPA was 1.4 times higher than in DOPA-treated monkeys. When binding in the lesioned areas was compared with the corresponding areas of the intact side various degrees of reduction were found. Although binding in the ventromedial areas of the lesioned side in untreated monkeys was slightly higher than in the corresponding areas on the intact side, this was not a consistent finding in DOPA-treated monkeys.

Electrophysiological studies of single striatal neurons

M3 and M4 were used for electrophysiological study. Single neuron activities in the caudate nucleus (112 neurons) and the putamen (293 neurons) of both sides were investigated. When the tip of the recording electrode was located in the dorsolateral caudate nucleus or putamen of the right side, i.e., in the area of the kainic acid lesion, no spontaneously active neurons were detectable. However, in the ventromedial striatum, which escaped the kainic acid lesion, recorded neurons exhibited normal activity, as in the left (intact) side. The putaminal neurons on the lesioned side (169 neurons) and on the intact side (124 neurons) were catagorized into two types (Kimura, 1986, 1987): (1) type I which were spontaneously active at about 2−7 Hz; (2) type II which had a very low spontaneous discharge rate (< 2 Hz) and showed phasic discharges time-locked to body movements.

Before L-DOPA administration, there was no right/left difference in neuronal discharge patterns in these parts of the striatum. However, when choreic movements occurred following the administration of L-DOPA, 53% (n = 49) of the recorded putaminal neurons in the ventromedial area began to show burst discharges which were detected

TABLE 5 TYROSINE HYDROXYLASE (T-OH) (nmol/mg PROTEIN/h)

Monkey		*C1*		*C2*		*M1*		*M2*		*M3*		*M4*	
		Intact side	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*
Rostral striatum													
Dorsolateral caudate	1	0.94	1.24 (+32%)	1.48	1.22 (−18%)	4.78	4.40 (−8%)	4.14	3.30 (−20%)	3.19	3.42 (+7%)	3.14	4.27 (+36%)
Dorsolateral putamen	2	2.13	2.03 (−5%)	1.48	1.54 (+4%)	4.57	4.63 (+1%)	4.62	5.19 (+12%)	2.45	3.41 (+39%)	3.65	5.94 (+63%)
Ventromedial striatum (incl. nucleus accumbens)	3	1.39	1.92 (+38%)	1.35	1.35 (0%)	3.06	3.01 (−2%)	2.52	5.18 (+106%)	2.88	4.94 (+72%)	3.21	4.80 (+50%)
Caudal striatum													
Body of caudate	4	1.55	1.33 (−14%)	1.25	1.04 (−17%)	2.58	4.32 (+67%)	1.72	3.45 (+100%)	2.92	2.89 (−1%)	1.72	3.17 (+84%)
Dorsal putamen	5	1.99	1.38 (−31%)	0.77	0.28 (−64%)	2.62	3.03 (−11%)	3.08	3.14 (+4%)	3.08	3.27 (+6%)	3.49	3.11 (−11%)
Ventral putamen	6	1.62	1.57 (−3%)	0.60	0.85 (+42%)	2.99	4.06 (+36%)	3.26	2.98 (−9%)	4.08	3.40 (−17%)	–	2.98
Substantia nigra													
Rostral part	9	1.46	1.33 (−9%)	1.14	0.70 (−39%)	1.16	1.79 (+54%)	0.84	0.73 (−13%)	2.45	4.95 (+102%)	1.68	2.87 (+71%)
Caudal part	10	2.98	1.87 (−37%)	2.11	0.96 (−37%)	1.84	4.00 (+117%)	3.26	2.72 (−17%)	5.44	6.60 (+21%)	4.37	4.33 (−1%)

TABLE 6 SPIROPERIDOL BINDING (pmol/mg PROTEIN)

Monkey		*C1*		*C2*		*M1*		*M2*		*M3*		*M4*	
		Intact side	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*	*Intact side*	*Lesion side*
Rostral striatum													
Dorsolateral caudate	1	0.37	0.34 (−8%)	0.52	0.42 (−9%)	0.26	0.14 (−47%)	0.32	0.19 (−39%)	0.34	0.17 (−49%)	0.34	0.28 (−17%)
Dorsolateral putamen	2	0.52	0.46 (−12%)	0.45	0.34 (−24%)	0.33	0.19 (−43%)	0.30	0.21 (−30%)	0.35	0.24 (−32%)	0.36	0.22 (−40%)
Ventromedial striatum (incl nucleus accumbens)	3	0.34	0.39 (+14%)	0.38	0.43 (+13%)	0.16	0.20 (+31%)	0.19	0.21 (+8%)	0.26	0.25 (−4%)	0.35	0.26 (−25%)

for approximately 5–6 h after L-DOPA administration and disappeared together with choreic movements (fig. 4). The burst discharges of some of these neurons were time-locked to the choreic movements (fig. 4). On the other hand, type I neurons did not

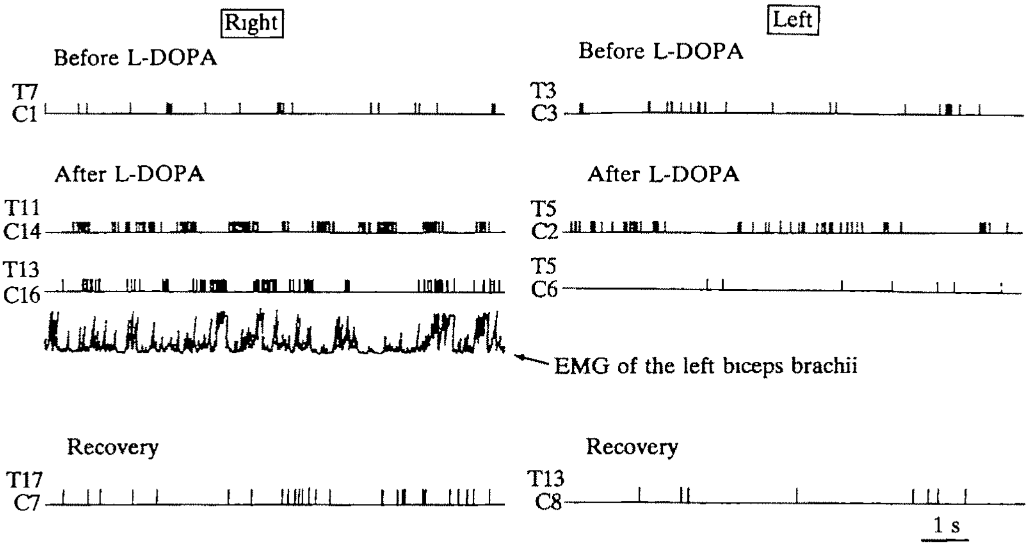

FIG. 4. Pen-recorded activity of single putaminal neurons on both lesioned (*right*) and intact (*left*) sides and muscle activity of contralateral arm to the lesioned striatum. Activity of many putaminal neurons was recorded before and 1 day after L-DOPA administration and also several days after L-DOPA as a recovery data. T and C on the left of the trace of neuronal discharges indicate number of track of microelectrode penetration and order of neurons encountered in a track, respectively. EMG of the left biceps brachii was recorded through rectification and leaky integration.

TABLE 7 FREQUENCY OF IMPULSE DISCHARGES OF THE PUTAMEN NEURONS (A) AND CAUDATE NEURONS (B)

A*	*Intact side*		*Lesion side*	
	Type I	*Type II*	*Type I*	*Type II*
Before L-DOPA	6.6±1.5 (n = 23)	1.5±1.7 (n = 30)	5.6±1.9 (n = 27)	1.7±1.4 (n = 27)
After L-DOPA	6.7±1.6 (n = 12)	2.6±2.3 (n = 35)	5.7±1.6 (n = 19)	4.5±3.0** (n = 73)
Recovery	6.0±2.2 (n = 5)	2.5±2.3 (n = 19)	6.9±1.6 (n = 11)	1.3±1.2 (n = 13)

B*	*Intact side*	*Lesion side*
Before L-DOPA	4.4±2.9 (n = 10)	3.4±2.4 (n = 19)
After L-DOPA	4.3±2.3 (n = 27)	3.4±2.7 (n = 25)
Recovery	3.2±1.8 (n = 17)	3.9±2.9 (n = 14)

* Values indicate frequency of impulse discharges in Hz (mean±SD), n = numbers of neurons, ** indicates that the value is statistically different from the value before L-DOPA ($P < 0.001$, Wilcoxon test)

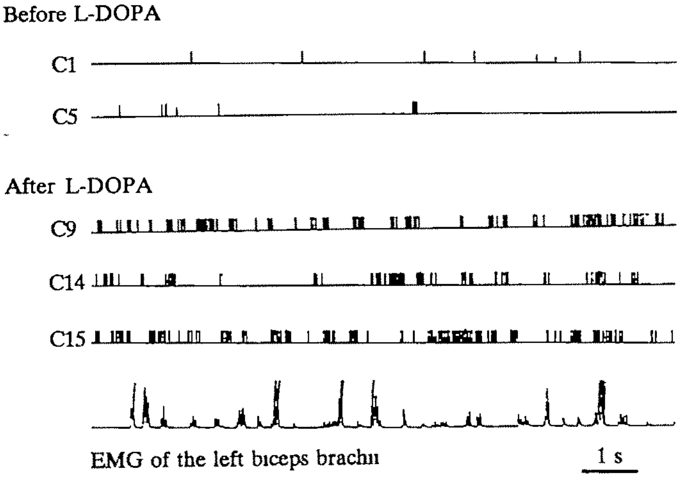

FIG. 5. Effect of deafferentation on the activity pattern of single neurons of the right putamen and left arm muscles during choreic movements after L-DOPA administration. Recordings were obtained 5 days after section of dorsal roots at the level of C4 to T2 in M4 on the left side. The proportion of neurons which showed burst discharges related to limb movements increased, and thus it was indicated that the deafferentation essentially did not affect the bursting pattern of putaminal neurons during choreic movements of the limbs.

change their discharge frequency even when the choreic movements occurred. The discharge frequency of type II neurons of the lesioned putamen increased from 1.7 ± 1.4 Hz (mean ± SD) before L-DOPA to 4.5 ± 3.0 Hz after L-DOPA administration, while that of type I neurons did not show significant changes (Table 7A). The discharge pattern of the putaminal neurons on the intact side was not significantly altered, although there was a slight increase of discharge frequency of type II neurons after L-DOPA administration. Although burst discharges of neurons in the right caudate nucleus were also found in the nonlesioned area, the population of such neurons was extremely small (Table 7B).

Effects of cervical dorsal root section on burst discharges of striatal neurons during choreic movements

As shown in Table 1, in M4 dorsal root section at C4-T2 was performed. In this monkey, the patterns of striatal neuronal discharge observed after L-DOPA administration were compared before and after dorsal root section. As shown in fig. 5, burst discharges were found in large numbers of striatal neurons in exactly the same way as before section.

DISCUSSION

Choreic and ballistic movements induced by striatal kainic acid lesions and L-DOPA administration

Characterization of involuntary movements. Abnormal choreic or ballistic movements occurred in the contralateral limbs of monkeys M2, M3, M4, M5, M7 which received unilateral striatal kainic acid injections and also oral L-DOPA. The EMG patterns associated with the abnormal movements confirm their choreic nature; there was no component of myoclonus or dystonia. DOPA-induced dyskinesia has also been reported previously in models of experimental parkinsonism in monkeys (Goldstein *et al.*, 1973). It is important, however, to point out that the monkeys in our study did not exhibit any features of parkinsonian behaviour or of supersensitivity in dopamine receptors following the kainic acid lesions in the striatum. The underlying mechanism of the choreic movements in the present study is therefore probably different from that in DOPA-induced dyskinesia.

Sax *et al.* (1973) also reported briefly the effects of L-DOPA administration on motor behaviour of monkeys with radiofrequency lesions of the caudate. The administration of L-DOPA was reported to produce 'stereotyped buccolingual dyskinesia' and 'flexion-extension movements of the fingers, toes, and wrists in a stereotyped manner'. This may be the first report to show the combined effects of striatal lesions and the administration of L-DOPA. Although it is clear that these monkeys did show 'oral dyskinesia', the description was not clear enough to regard the stereotyped movements of the limbs as 'choreic movements'. More recently, Ohmoto *et al.* (1981) briefly described a monkey with unilateral surgically-induced destruction of the caudate nucleus which showed occasional oral dyskinesia but did not show any dyskinetic movements of the limbs after the oral administration of L-DOPA (100–150 mg L-DOPA with 10–15 mg MK486). In contrast to these rather conflicting previous reports, the present study shows that consistent choreic movements can be induced by the combined effects of striatal lesions and the administration of L-DOPA. One of the differences beween the present study and the two previous reports may be in the method used to lesion the striatum. The use of kainic acid preserves the afferents to the striatum, whereas surgical lesions destroy them. Preservation of dopaminergic afferents to the striatum may be especially important for the generation of choreic movements in experimental monkeys (*see below*).

Relationship between choreic movements and the size of the striatal lesion. The lesions in monkeys which showed choreic movements had various sizes and locations. M1 and M6, whose striatal lesions were large enough to occupy more than 60% of the rostral striatum especially of the putamen, did not show choreic movements following the administration of L-DOPA. On the other hand, M4, whose rather small striatal lesion was located exclusively in the rostral part, did show choreic movements after L-DOPA administration. It therefore seems that partial (probably less than 60% in volume) lesions of the rostral part of the striatum are necessary for the generation of choreic movements. In other words, preservation of some part of the striatum is necessary.

Relationships between choreic movements and neurotransmitter markers in the basal ganglia

GABAergic markers. GABAergic neurons in the striatum send long axons to both segments of the globus pallidus and the substantia nigra (Yoshida *et al.*, 1972). They also have short axon collaterals terminating in the striatum (Kitai, 1981). In general, GABA concentrations in the ipsilateral globus pallidus, dorsolateral striatum and substantia nigra decreased on the lesioned side, as expected from previous lesion studies (Kim *et al.*, 1971). Moreover, it is noteworthy that the size of the rostral caudate and putaminal lesions approximately paralleled the extent of decrease of GABA in the globus pallidus and the substantia nigra. However, the size of the caudal striatal lesions did not parallel the magnitude of the decrease of GABA in its projection areas. It is difficult to explain this discrepancy in the light of anatomical knowledge concerning topographical arrangements of the striatopallidal and the striatonigral fibre connections. The method used in the present study to dissect the tissue for sampling may not be precise enough to clarify the topographical fibre connections of GABAergic neurons.

Monkey M1 exhibited the greatest overall decrease in GABA concentrations in the rostral striatum and in the sustantia nigra among 4 monkeys which received L-DOPA, but M1 did not exhibit choreic movements. There was thus no clear correlation between choreic movements and the decrease of GABA in the basal ganglia. It is assumed in general that when the inhibitory neurotransmitter GABA decreases in the substantia nigra, nigral dopaminergic neurons may be disinhibited. This may be a plausible mechanism for the generation of choreic movements in Huntington's disease (Waddington and Cross, 1978), but the present results do not support this assumption. GABA may not be the sole agent responsible for the generation of choreic movements. However, our results do not exclude the possibility that a decrease of GABA in the basal ganglia may reset the nigral neurons towards an 'activated' state and contribute to the generation of choreic movements.

Cholinergic markers. ChAT activity is localized in the cholinergic interneurons of the striatum (Kimura *et al.*, 1979; Kanazawa *et al.*, 1985). A significant decrease of ChAT activity in the kainic acid injected areas of the striatum is therefore easily explained as a direct result of the loss of cholinergic interneurons in that area. Since alterations of ChAT activities in the nonchoreic monkey M1 were essentially the same as those in the choreic monkeys M2, M3 and M4, there appeared to be no correlation between the generation of choreic movements and the change in ChAT activities.

Dopaminergic markers. As markers of the nigrostriatal dopaminergic neurons, tyrosine hydroxylase (T-OH) activity and spiroperidol binding were determined in the present study. T-OH activities in the striatum, but not in the substantia nigra, of the intact side of monkeys treated with L-DOPA (M1, M2, M3, M4) were significantly higher than those of monkeys not treated (C1, C2).

T-OH is a rate-limiting enzyme in catecholamine synthesis and is controlled by a negative feedback mechanism by its end products (Nagatsu *et al.*, 1964). Indeed, in experiments on rats with unilateral nigrostriatal 6-hydroxydopamine lesions, Melamed *et al.* (1980) demonstrated a slight but definite *decrease* of T-OH activity in the striatum of the *intact* side following a single dose of L-DOPA or L-DOPA with carbidopa. On the other hand, Dairman and Udenfriend (1971) and Takashima *et al.* (1987) found no alteration of T-OH activity in rat brain or striatum after long-term L-DOPA administration. It is difficult to interpret the discrepancy between these reports and ours at present.

A most prominent finding which seemed to have a clear correlation with the generation of choreic movements was the significant increase (more than 50%) of T-OH activity in the rostral ventromedial striatum on the lesioned side which escaped the kainic acid lesion in choreic monkeys (M2, M3, M4) as compared with the corresponding area on the intact side. The increase of T-OH activity in the undamaged rostral ventromedial striatum relative to the intact side was also observed in C1, whose lesion of the rostral striatum was approximately the same size as those of choreic monkeys, but not in C2 whose lesion was smaller in size, involving less than 25% of the rostral striatum. These results suggest that the increase of T-OH activity in the unlesioned ventromedial striatal area occurred only when the rostral striatal lesion was of appropriate size, namely more than 25% and less than 60%.

In contrast to the marked increase of the *presynaptic* marker of the nigrostriatal dopaminergic system in the undamaged area of the striatum, the *postsynaptic* marker, spiroperidol binding did not show any significant alteration. Judging from the finding that spiroperidol binding in the striatum of the *intact* side was slightly decreased in monkeys treated with L-DOPA when compared with untreated animals, a possible increase of spiroperidol binding in the undamaged area of the striatum might have been cancelled by the administration of L-DOPA.

It is therefore reasonable to speculate that when kainic acid was injected into the rostral striatum, the presynpatic dopaminergic terminals, rather than the postsynaptic dopamine receptors, located in the remaining striatal area, might be activated. When monkeys receive a systemic administration of L-DOPA, dopaminergic terminals in the remaining area which are preactivated by the kainic acid injection are even further activated. It is not certain at the moment, however, whether the remaining area should really be located in the ventromedial striatum for the generation of such activation. Further study is required to answer this question.

Agid *et al.* (1973) similarly demonstrated that partial destruction of the nigrostriatal dopaminergic neurons in the rat by the injection of 6-hyroxydopamine into the substantia nigra caused hyperactivity of the remaining nigral neurons. They suggested that 'this may represent a compensatory mechanism induced by a deficiency of dopamine at some postsynaptic receptor sites. An interneuronal feedback process mediated through a direct or polysynaptic striato-nigral connexion is probably involved in this effect'. Andersson *et al.* (1980) found that kainic acid injections into the striatum of the rat caused an increase of dopamine levels in the medial part of the striatum. They suggested that 'this increase of dopamine in the striatum seems to reflect a functional response of the dopaminergic nerve terminal to an indirect intrastriatal dopamine release caused by kainic acid and/or to a loss of inhibitory striato-nigral feedback mechanisms after degeneration of striatal neurons'. Taking these previous findings into consideration, we can consider at least the possible mechanisms for the increase of T-OH activity in the unlesioned striatal area. (1) Since the striatonigral GABAergic inhibitory inputs are partially reduced by kainic acid, the dopaminergic nigral neurons may be disinhibited, i.e., activated. (2)

Certain populations of varicosities on nerve terminals of the nigrostriatal fibres are involved in the kainic acid lesion and lose their partner (dopamino-receptive) neurons. Another population of varicosities belonging to the same fibres may be located in the ventromedial striatum and not involved in the kainic acid lesion. If this is the case, compensatory activation of the dopaminergic component may occur in the undamaged area because of the loss of partner cells (dopamine-receptive cells) in the lesioned area through an 'axon reflex-like' mechanism. (3) Since there is evidence for dendrodendritic interconnections between dopaminergic nigral neurons (Cheramy *et al.*, 1981), the nigral cells innervating the undamaged area of the striatum receive activating influences through dendrites from another group of nigral cells innervating the lesioned area, in which dopaminergic terminals have lost their partner cells in the striatum. We have no evidence at the moment to determine which is the most plausible possibility. However, the first is an unlikely possibility in our monkeys because there was no clear correlation between a decrease of GABA in the substantia nigra and an increase of T-OH activity in the undamaged area of the striatum. In any case, the present results strongly suggest that for the induction of such a dopaminergic activating process, more than about 40% and less than 75% of 'seemingly normal unlesioned' striatal tissue seems to be necessary.

As for the relationship between choreic movements and the dopaminergic 'activated' process, it is reasonable to assume that certain populations of the 'activated' nigrostriatal dopaminergic neurons which innervate the unlesioned area of the striatum may not alone be sufficient to produce spontaneous choreic movements. Since L-DOPA can cause general activation of dopaminergic components, those nigrostriatal dopaminergic nerve terminals in the unlesioned area of the striatum which are preactivated may be more likely to attain a level of activity sufficient to produce choreic movements in the contralateral limbs.

Effects of apomorphine or methamphetamine. Apomorphine predominantly stimulates postsynaptic dopamine receptors (Andén *et al.*, 1967), while methamphetamine enhances the presynaptic release of dopamine (Creese and Iversen, 1975). The effect of methamphetamine on monkeys with a unilateral kainic acid lesion in the striatum was essentially the same as that of L-DOPA, whereas the effect of apomorphine was not. This supports the above-mentioned interpretation of the biochemical results, that the 'activated' dopaminergic components in monkeys would be the *presynaptic* dopaminergic nerve terminals rather than the *postsynaptic* dopamine receptors.

Burst discharges of striatal neurons during choreic movements

In the present study, a considerable number of neurons in the ventromedial putamen which escaped the kainic acid lesion showed burst discharges when L-DOPA was administered and choreic movements appeared in the limbs contralateral to the kainic acid lesion. The burst discharges of some recorded neurons were time-locked to the choreic movements (fig. 4). The burst discharges of neurons in the ventromedial putamen may therefore play a key role in generating choreic movements. Although the burst-generating mechanism in striatal neurons may differ between DOPA-induced choreic movements and voluntary movements, the neuronal pathway involved could be the same.

The first and principal one is the striato-pallido-thalamo-cortical loop and cortico spinal path, and the second the striatopallidal or striatonigral path with a descending path from the globus pallidus and/or the substantia nigra to the brainstem-spinal cord through the tegmental area.

The activity of many neurons in the primate putamen is modulated by passive somatosensory manipulation of peripheral body parts (Crutcher and DeLong, 1984; Liles, 1985). However, the capacity of peripheral somatosensory afferents to modulate or even to generate burst discharges of striatal neurons seemed to be negligibly small during the choreic movements induced by L-DOPA administration in this study, since deafferentation by dorsal root section had no effect.

As discussed above, increased activity of dopaminergic terminals may exist in the undamaged ventromedial striatum. It was in this part of the striatum where type II neurons with burst discharges were recorded. These observations therefore permit us to hypothesize that an excess of dopamine causes abnormal burst discharges of type II striatal neurons. Although the dopaminergic neurons in the substantia nigra have been demonstrated to give a monosynaptic EPSP on the striatal neurons (Kitai *et al.*, 1976), the action of dopamine on striatal neurons is still controversial. There are essentially two possibilities whereby dopamine could cause burst discharges in striatal neurons. One is by a direct action on the striatal neurons; the other is by disinhibition of local inhibitory circuits, such as through the recurrent collaterals of projection neurons or through inhibitory interneurons. In this context, it is worth noting the following observations. (1) Medium-sized spiny neurons in the striatum have been demonstrated to have a calcium component in their action potential as well as a sodium component and to show high frequency action potentials following small depolarizing currents (Kita *et al.*, 1985), although the projection neurons in the striatum (type II) have very low spontaneous discharge rates (Kimura, 1987). (2) Injection of small amounts of GABA antgonists into the putamen induces choreiform dyskinesia in the monkey, although single neuron activity was not examined (Crossman *et al.*, 1987).

Similarity and dissimilarity in underlying mechanisms between choreic movements in experimental monkeys and those in Huntington's disease

The aim of the present study was to produce choreic movements in monkeys in order to shed light on the neural mechanisms of human choreic movements, especially those of Huntington's disease. It will therefore be relevant here to clarify similarity and dissimilarity of choreic movements with those of Huntington's disease.

Concerning a pathophysiology of choreic movements in Huntington's disease, Bruyn and Went (1986) recently summarized previous reports and stated that the choreic movements in Huntington's chorea result from 'either (a) hypersensitive striatal dopamine receptors or (b) increased striatal dopamine synthesis, release or turnover'. Moreover, they stated that 'inasmuch as the striatal content of dopamine does not seem to be increased (in an absolute sense) in Huntington's chorea' (Bernheimer and Hornykiewicz, 1973), choreic movements 'must result from an abnormal receptor response of the transmitter-recipients rather than from increased dopamine synthesis and release'. However, Melamed *et al.* (1982) and Reisine *et al.* (1978) demonstrated a marked decrease, rather than an increase of dopamine receptor density in the striatum of Huntington's disease. In addition, Bird *et al.* (1980) revealed an increase of dopamine concentration (per unit protein) and of T-OH activity in the striatum of Huntington's disease. Since the striatum shows severe atrophy in Huntington's disease, the total amount of striatal dopamine and T-OH activity should be reduced. However, it is reasonably assumed that the magnitude of the reduction of dopamine is much less than that of dopamine receptors. The observed facts therefore favour the hypothesis that choreic movements in Huntington's disease result from a relative or absolute excess of dopamine in the striatum rather than dopamine receptor supersensitivity. This hypothesis is closely similar to that proposed here in the experimental monkeys.

The situation in the monkeys which exhibited choreic movements following L-DOPA administration may be similar to that observed in at risk cases of Huntington's disease. According to Klawans *et al.* (1980), among 10 at risk individuals who exhibited choreic movements following the provocative administration of L-DOPA, 5 were found to be clinically affected in an 8-year follow-up study. Although this is usually interpreted as dopamine receptor supersensitivity but is now precluded as a predictive test, these results are also consistent with the hypothesis of presynaptic dopamine hyperactivity.

The most important dissimilarity between choreic movements in Huntington's disease and those in monkeys lies in the spontaneity and duration of choreic movements. Choreic movements in monkeys never occurred spontaneously and did not last more than 6 h, even after L-DOPA administration, whereas choreic movements in Huntington's disease occur spontaneously and usually last until the end stage of the disease. A much longer follow-up period after kainic acid or quinolinic acid lesions without L-DOPA administration is needed in the primate model.

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FURTIVE INCURSIONS INTO BICAMERAL MINDS

INTEGRATIVE AND COORDINATING ROLE OF SUBCORTICAL STRUCTURES

by JUSTINE SERGENT

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SUMMARY

Section of forebrain commissures results in a pervasive disconnection syndrome characterized by the incapacity of the two cerebral hemispheres to exchange their respective information content. Yet commissurotomized patients are capable of unified behaviour in their daily activities and, in laboratory situations, are able to produce a single response as a function of the information received by both hemispheres This study further investigated the nature of the information that can be communicated between the disconnected hemispheres in 3 commissurotomized patients. The first part comprised 7 experiments in which the patients were required to compare 2 digits simultaneously, 1 projected to each hemisphere. While the stimulus and experimental conditions were the same across experiments, the information content on which to base the comparison was manipulated. The level of performance varied as a function of the information content, such that the patients were unable to decide whether the 2 digits were the same or different, performed better when required to compare the digits in terms of their odd-even category, and were highly accurate at deciding which digit was the higher. In addition, when instructed to focus on the numerical value of the digits to decide whether the 2 digits were the same or different, they performed significantly above chance. In the second part, they were requested to report verbally information about emotional faces and familiar faces projected to the right hemisphere While they were unable to access the visual characteristics and the identity of the faces, they could provide various correct items of semantic information about the individuals. Taken together, the results indicate that the disconnection syndrome is limited to specific categories of information and does not extend to all the knowledge that may be derived from a given object. They also suggest that subcortical structures do not serve simply as pathways linking the two hemispheres but play an essential coordinating role in the integration of hemisphere activity.

INTRODUCTION

The section of forebrain commissures for the relief of intractable epilepsy has provided a unique opportunity to study the functional capacities of the brain in the absence of the main means of communication between the two hemispheres. Besides the therapeutic benefits of such a surgical procedure (Bogen and Vogel, 1975; Andermann *et al.*, 1988), the most striking outcome of the commissurotomy has been a pervasive disconnection syndrome (Bogen, 1985) suggestive of the coexistence of two independent cognitive systems within the same brain, each being essentially unaware of the information and operations going on in the other.

However, another outcome of the commissurotomy, much less striking but none the

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less important and significant, is that, would it not be for carefully controlled experiments, the behaviour of the patients would offer almost no clue as to their unusual neurological condition, and they display hardly any sign, outside the laboratory, of their having 'two separate conscious entities or minds running in parallel in the same cranium' (Sperry, 1966–1967). If the commissurotomy prevented all communication between the two hemispheres, either they would be competing for the control of behaviour, which would result in confusions, dissociations or hesitations, or one hemisphere would take control of behaviour, which would result at least in neglect of one side of the body and extrapersonal space (e.g., Joynt, 1977). This is not the case, and commissurotomized patients appear to act as unified individuals with purposeful behaviour, suggesting some capacity of the 'split brain' to join and integrate the resources of its two hemispheres. This apparent normality of the behaviour of commissurotomized patients, in the context of an otherwise well demonstrated hemisphere disconnection, raises questions about the functioning of the 'split brain' and the processes by which unified behaviour and, therefore, hemisphere integration, can be achieved.

At least three mechanisms could account for the integration of behaviour in commissurotomized patients: (1) duplication of information in the two disconnected hemispheres, for instance through eye and head movements (Sperry, 1984); (2) cross-cueing strategies whereby one hemisphere elaborates and emits some signals picked up by the sensory organs projecting to the other hemisphere (Gazzaniga and LeDoux, 1978; Bogen, 1986); and (3) subcortical pathways between the hemispheres, left undivided by the commissurotomy and providing a common stem to the two sides of the brain (Sergent, 1986). While each of these mechanisms may contribute to the unified behaviour of commissurotomized patients outside the laboratory, experiments have been designed in which the influence of the first two mechanisms was eliminated, thus providing a method for assessing the role of subcortical structures in the functioning of the commissurotomized brain.

Results from such experiments, whereby performance of one hemisphere is assessed as a function of the information received by the other hemisphere, have shown that some exchange of information across the hemispheres could be achieved and that subcortical structures could mediate interhemispheric transfer, albeit with considerably less efficiency than forebrain commissures. Such subcortically mediated interhemispheric transmissions have been observed for general emotional tone (Gazzaniga, 1970), ambient visual perception (Trevarthen and Sperry, 1973), orientational and attentional cues to locations in the other visual field (Holtzman *et al.*, 1981; Holtzman, 1984), or crude semantic information (Zaidel, 1981; Cronin-Golomb, 1986). Myers and Sperry (1985) have regarded this evidence as suggesting that 'subcortical transmissions are largely connotative, contextual or orientational in nature' but are insufficient to bring together most of the information content of each hemisphere.

There are, however, reasons to believe that subcortical structures may transmit more than a quantitatively and qualitatively limited amount of information and may do more than serving as interhemispheric pathways. First, the type of information postulated by Myers and Sperry (1985) as being transmitted subcortically would be insufficient to mediate integrated behaviour which requires more than 'connotative' or 'orientational' information to be produced. Secondly, when commissurotomized patients are presented simultaneously, in each visual field, with information associated with conflicting responses, they are able to respond as a function of a preestablished criterion, suggesting that the two disconnected hemispheres have the capacity to cooperate in resolving the conflict, which requires some coordination of their activity, presumably at a subcortical level (MacKay and MacKay, 1982; Sergent, 1983, 1986). Thirdly, recent empirical evidence suggests that the commissurotomized brain is capable of well-adapted and appropriate unified responses to information initially segregated between the two hemispheres, even in conditions involving higher-order processing such as calculation or lexical decision (Sergent, 1987). In addition, evidence from patients born without a corpus callosum indicates minimal loss of interhemispheric integration, suggesting that midbrain structures may compensate for the absence of the main means of communication between the hemispheres (Jeeves, 1965, 1979). Thus subcortical coordination and integration of hemisphere operations may underlie the behavioural coherence displayed by commissurotomized patients in their daily activities, allowing them to relate different parts of the visual fields, to combine the outcome of the operations respectively carried out by each hemisphere, and to maintain a unity of purpose in their action.

One important yet paradoxical aspect of these findings was the joint occurrence of a unified response to, but a disunified awareness of, the information initially divided between the two hemispheres (Sergent, 1987). That is, the patients were able to display unity and coherence of behaviour even though each hemisphere was unaware of the information received by the other, the latter finding concurring with the main feature of the disconnection syndrome—the presence of separate left and right domains of conscious awareness. The commissurotomy thus results in apparently contradictory outcomes depending on whether awareness of information or production of behaviour is the relevant criterion.

This raises two central questions in the evaluation and understanding of the functioning of the 'split brain'. Which of these two criteria is the more appropriate to assess interhemispheric communication? Why is each hemisphere unaware of the information content of the other when the commissurotomized brain appears to act as a function of the information content of both hemispheres? It is perhaps noteworthy that the first question would be irrelevant if the problem were to be examined in nonhuman animals. While humans can express their mental contents through language, in all other animals mental or information content are inferred only from somatic motor behaviour. If a 'split-brain' animal were to respond in an accurate and unified manner to a bilateral stimulus presentation, as did the human commissurotomized subjects, it would be regarded as evidence that the two hemispheres were capable of exchanging their respective information. A motor behavioural response would be sufficient proof *for* the combination of information content across the hemispheres and *against* hemisphere disconnection. Therefore, when a patient makes a unified motor response but is unable to tell, with the left hemisphere, what information appeared in the left visual field, one may speak of unawareness of this information but not necessarily of absence of this information in the left hemisphere. A verbal response is only one among several methods of assessing whether or not the left hemisphere holds a specific information content and, being the end-stage of highly elaborated processing, its failure of being emitted may not exclude the existence of less elaborated information. It is therefore of significance that much, but not all, of the evidence in support of the disconnection syndrome relied on the use of a verbal response, and that most of the findings suggestive of subcortical transmission were also obtained through a verbal response (Gazzaniga, 1970; Trevarthen and Sperry, 1973; Zaidel, 1981; Myers and Sperry, 1985; Cronin-Golomb, 1986). In both cases, the evaluation of interhemispheric transfer may have depended on too restrictive a criterion that may not have exhausted all the manifestations of information content within one hemisphere. In response to the first question, it may thus be suggested that a somatic motor response and a verbal response are two operational criteria that tap different levels of functioning, the former providing a broader sample of information content than the latter.

It may then be necessary to consider the relation beween cerebral processing and information content in order to address the second question, that of unawareness of information used in making a decision. There is growing evidence that any mental process, whether the production of manual response to a numerical stimulus (Gevins *et al.*, 1987) or the learning of a two-shape discrimination (John *et al.*, 1986), leaves almost no cerebral space uninvolved, at least within a modality. What is considered a unitary mental operation requires the activation of many cortical and subcortical areas, and it is reasonable to assume that the diversity and multiplicity of cerebral areas activated do not correspond to all that the subject is aware of in a given mental operation. Not only are many cortical and subcortical areas simultaneously activated in such processes, but the pattern of activation unfolds through a series of stages not all of which are accessible to awareness, and mental contents appear to be a complex integrative function of neural events. Consider the example of face identification. A face can be identified by its name in less than one second, yet the naming of the face is the end product of many operations that were necessary to narrow down the response possibilities until one was chosen (Sergent, 1989). In providing the name of a face, the subject is not aware of all these operations, but a considerable amount of information had to be activated, representing associated knowledge about this face. Even if this information does not reach consciousness, it can be shown that it is in an active state, as suggested by the patterns of interference and facilitation in experimental tasks that examined the influence of this information on face recognition (Bruce and Young, 1986). There is, therefore, more information being activated than a subject may be aware of, and some of this information may be sufficiently active to determine behaviour even if it is not codified in a sufficiently specific manner to be amenable to verbal utterance.

These considerations have several implications in the present context: (1) a given stimulus may activate a variety of information representing different levels and categories of knowledge about this stimulus; (2) not all levels of knowledge may be equally accessible; (3) unawareness of information does not necessarily imply absence of this information; and (4) information of which the subject is unaware may nonetheless contribute to the production of behaviour. As a consequence, the occurrence of interhemispheric transfer in commissurotomized patients may depend on a series of factors such as the nature of the information content to be communicated, the nature of the tasks demands and their respective purposes, not all of which have been taken into account in the evaluation of the disconnection syndrome.

The aim of the present study is thus two-fold. On the one hand, it examines the validity of the foregoing suggestions and attempts a better specification of the nature and extent of the information being transferred between the two disconnected hemispheres. By considering patterns of dissociations and parallel effects across tasks as a function of the respective informational demands of each task, and as a function of the motor verbal or somatic response, inferences can be made about the contents that are accessible across hemispheres and the appropriateness of the coding of these contents for verbal responses. On the other hand, the pattern of results, along with dissociations between unified response and unawareness of information, may provide some clues as to the structural and functional organization of information respresentations underlying the production of behaviour.

The experimental investigation is divided into two parts. The first part consists of an investigation into the disconnection syndrome which typically implies an inability of commissurotomized patients to compare information content held in each hemisphere. A series of experiments is carried out, involving cross-comparison of information between the hemispheres and using exactly the same stimuli throughout but requiring different informational demands to perform the comparison. The second part examines the selectivity of interhemispheric transfer with respect to facial information.

EXPERIMENTAL INVESTIGATION

All the tasks involved lateral tachistoscopic presentations, either unilateral or bilateral and, except when indicated otherwise, they were conducted in the order they are reported. Most of the tasks required a manual-timed response that could be initiated by either hemisphere and that compelled the patients to make a quick decision, precluding cross-cueing strategies.

General methods

Subjects

The subjects were 3 commissurotomized patients, a 54-yr-old womam (N.G.), a 37-yr-old man (A.A.) and a 35-yr-old man (L.B), who had undergone section of the corpus callosum and the anterior hippocampal and habenular commissures (as well as a large massa intermedia in N.G. and the right fornix in L.B.) in 1963, 1964 and 1965, respectively. They have been extensively tested since their operation, N G. and L.B. more than A.A., and they have displayed the typical disconnection syndrome (Bogen, 1985). Details of their surgical and neurological histories have been reported repeatedly (Bogen and Vogel, 1975; Sergent, 1986) They are under anticonvulsant medication and, while N G. has had no definite seizures since 1972, both A.A and L.B. still have minor attacks during sleep or on waking. Recent investigation by magnetic resonance imaging (MRI) has confirmed complete section of forebrain commissures in N.G (Bogen *et al.*, 1986) and A.A. (J. E Bogen, personal communication, October 1987). L.B 's brain has been subjected to MRI scanning on two different occasions, and the radiologist's reports indicate complete section of forebrain commissures. However, on both occasions, a small patch of filmy substances was detectable in the centre of the splenium of the corpus callosum. Given the location of the patch and the fact that the whole perimeter of the splenium was reached during the operation, this is not thought to reflect spared callosal fibres. The patients were tested in October 1987, but the experiments on faces have been carried out every year since 1984.

Equipment

The stimuli were presented via a Gerbrands tachistoscope and were rear-projected onto a translucent screen located 57 cm from the subject's eyes. A response panel containing a central knob and 2 lateral Morse keys was placed in front of the subject The Morse keys were located 17 cm on each side of the central knob. The subject rested the middle finger of the responding hand on the knob and moved this hand toward 1 of the 2 keys to make a response. Stimulus onset triggered an electronic timer (Gerbrands, model 130) which was stopped when the subject pressed 1 of the 2 keys. Both speed and accuracy of responses were recorded.

Procedure

The same procedure was used in most tasks and its main characteristics are described here. The subjects were tested in a private room, seated in front of the screen with the head supported by a chin-rest so that the eyes were at the level of the central fixation point The subjects were requested to hold their legs and feet apart, to keep their teeth tight and the lips closed, and the nonresponding hand lay on the side of the table with no contact with the response panel, in order to avoid any potential transfer of information between the two sides of the body.

A trial started with the signal 'ready' by the experimenter to warn the subject to fixate on the central dot. About 1 s later, a stimulus display was presented for 150 ms, containing information either in one visual field or in both visual fields, and the subject was required to take a manual response, according to the instructions, by moving the hand from the resting position to the appropriate key. Both speed and accuracy were stressed in the instructions, and the subjects were reminded from time to time of the importance of fixating the central point, two procedures with which they are highly familiar Each experiment comprised several sessions preceded by practice trials. The responding hands were counterbalanced across sessions. Order of stimulus presentation within each session was random.

Except for the tasks with faces (Tasks 8, 9), the stimuli appeared as white on a black background, 7.5° from fixation in the left and/or the right field and they subtended 3° of visual angle horizontally. An independent observer monitored the subjects' eyes to control for eye movements. To ensure that the subjects were fixating as requested, 'surprise' trials were intermixed within the experimental trials, at random intervals. They consisted of the presentation of a single letter in the right visual field, appearing 1° from fixation and subtending 0.28° of visual angle, and the task of subject was to name the letter. The letters were of such a size that they could not be identified if they fell more than 2° from the fovea. The subjects' capacity to name the letters would thus be a guarantee that their eyes did not deviate more than 1° from fixation within the right or the left visual field and that the lateral presentations of the experimental stimuli effectively resulted in projection to the contralateral hemispheres. All 3 patients were accurate in reporting the 'surprise' letters in all experiments, confirming that they could be relied upon to follow the instructions. After each task, 20 additional trials were conducted and the subjects were requested to describe what they saw, on 10 trials without making a decision and, on the other 10 trials, after making a decision. Across all tasks of Part I, neither A.A. nor N.G. were ever able to report the information appearing in the left field, while L.B. could do so after a delay varying between 2 and 5 s.

Part 1: Interhemispheric comparison

When presented with 2 stimuli, 1 in each visual field, commissurotomized patients typically cannot tell whether the stimuli are the same or different (Sperry *et al.*, 1969; Gazzaniga, 1970; Sergent, 1986), and this constitutes one of the most convincing demonstration of the disconnection syndrome. Yet it may appear puzzling that the commissurotomized brain would be able to combine and integrate the information of its two hemispheres (Sergent, 1986, 1987) but would not be able to compare the information held respectively by each hemisphere. The purpose of this part of the study was to examine some of the factors that may account for this divergence, along with specifying more satisfactorily the nature of the information that can be transferred for comparison.

From a theoretical standpoint, there is a fundamental difference between a task aimed at interhemispheric integration and a task aimed at interhemispheric comparison. Interhemispheric integration is a basic function of the brain by which the two cerebral hemispheres join their resources and coordinate their respective operations for the production of unified behaviour. Both the corpus callosum and subcortical structures normally play a critical role in this integration and, in the commissurotomized brain as well as in the acallosal brain, subcortical structures alone are left to play this role. By contrast, interhemispheric comparison may be a powerful diagnostic tool for hemisphere disconnection but, in so far as the two hemispheres are complementary, not duplicate, processors, they do not need to examine whether they carry the same information content. Although this distinction may be somewhat academic, it nonetheless suggests that integration and comparison make different demands on the brain in terms of exchange of information. Thus the integration task is orientated towards action and the information content is processed with the purpose of producing and regulating behaviour. The comparison task is primarily a test of mental performance, involving a reciprocal check of information content. In addition, the comparison task may have used a very stringent criterion for the evaluation of interhemispheric communication. The cross-comparison is typically performed in terms of stimulus identity, which involves a match of very specific information that uniquely defines each stimulus to determine whether it is the same or different from others. However, as pointed out earlier, when accessing the identity of an object, associated information is also activated, and this information may be communicated to the other hemisphere even if the actual identity of the object is not. Thus while the corpus callosum may be required to transfer fine visual information, associated knowledge about the stimulus, which does not require a one-to-one mapping of the component information and which may be more widely distributed and represented within the cortical and subcortical network, may be more readily amenable to interhemispheric exchange.

This part of the study was therefore designed to examine how different information content derived from the same set of stimuli (the 9 digits) influences the efficiency of interhemispheric transfer in comparison tasks.

Task 1

This experiment tested the capacity of the patients to decide whether or not 2 digits, 1 appearing in each visual field, were the same. Although there is much evidence indicating that commissurotomized patients are unable to perform such a task, one recent experiment has shown that, under certain conditions, successful cross-comparison can be achieved. Johnson (1984) reported that N.G. was able to match 2 letters or 2 digits, 1 being presented in each visual field. However, accuracy was a function of the retinal eccentricity of stimulus presentation and of the number of items comprising the stimulus set: a relatively large stimulus set, or a relatively large retinal eccentricity, resulted in chance responding. For instance, N.G was unable to compare letters chosen from a set of 8, but she could do so when fewer letters comprised the stimulus set In the present study, the stimulus set comprised 9 digits, and they were presented at a retinal eccentricity that would have yielded chance responding according to Johnson's (1984) study.

The stimuli were the digits of 1–9. At each trial, 1 digit appeared in the right visual field and 1 in the left visual field, at 7.5° from fixation (*see* figs 1.1, 1.2). There were 36 pairs of 'different' digits and 9 pairs of 'same' digits. Each 'same' pair was mounted on 4 slides, such that there was an equal number of 'same' and 'different' pairs. A.A. was tested in 4 experimental sessions and L.B. and N.G. in 6 experimental sessions, half of the sessions with each responding hand. Within each experimental session, 8 'surprise' trials were intermixed. The subjects were instructed to decide whether the 2 digits were the same or different and to respond by pressing one key or the other manually

Results. The results are shown in the left of Table 1, as a function of responding hand and they reveal individual differences among patients. Neither A.A. nor N.G. could compare the digits across the visual

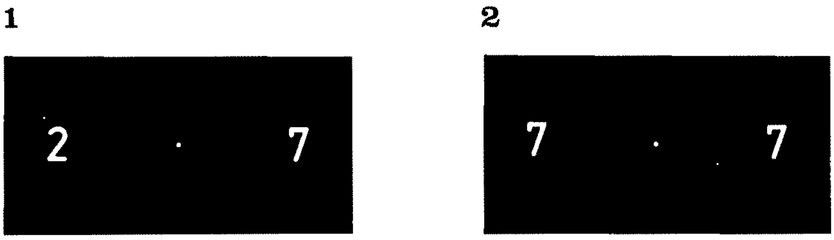

FIG 1 Sample of the stimulus displays used in Tasks 1, 2, 4–7. The comparison differed in each task (1) Are the two digits the same or different? (2) Are the two digits of the same category (both odd or both even) or of different category (one odd and one even)? (4) Press on the side of the higher digit (only 'different' pairs of digits were used). (5) Press on the side of the higher digit and raise both hands if they are equal (both 'same' and 'different' pairs of digits were used). (6) Press one key if one digit is higher than the other, and the other key if they are equal. (7) Same as Task 1.

TABLE 1 CORRECT MEAN REACTION TIMES (RT) IN ms, SEM, AND PERCENTAGE OF CORRECT RESPONSES FOR THE 3 SUBJECTS IN TASKS 1 AND 2

		Task 1		*Task 2*	
		Left hand	*Right hand*	*Left hand*	*Right hand*
A.A.	RT	1869	1643	1972	1841
	SEM	27 11	23.08	56.15	51.13
	% correct	54.17*	48.61*	55.5*	46.7*
L B	RT	1016	940	1297	1206
	SEM	15.91	13.73	28.82	26.03
	% correct	70.4	75 0	84 4	88.9
N.G.	RT	1326	1270	1612	1580
	SEM	17.39	20.40	50 09	52 38
	% correct	45.4*	51.9*	66.7	73.3

* Not significantly different from chance.

fields, and their responses were no better than chance in all conditions (χ^2 (1) <3, $P > 0.05$). Although they both responded faster with the right than with the left hand, this difference was not significant (F $<$ 1.5) On the contrary, L.B. performed significantly above chance (χ^2 (1) $= 35.85$ and 54, $P < 0.001$, for the left and right hand, respectively) and, although his performance was far from perfect and inferior to that of normal subjects in similar conditions, he was nonetheless capable of comparing the information held by the two hemispheres.

The results of both A.A. and N.G. conformed to the predictions of the disconnection syndrome and confirmed the inability of subcortical structures to mediate interhemispheric transfer of pattern information. The performance of N.G. departs from the finding reported by Johnson (1984) that she was able to cross-compare alphanumeric characters, and the present results suggest that, at least for N.G., some factors related to retinal eccentricity and/or size of the stimulus set may influence interhemispheric transfer. The results of L.B., however, are not consistent with the disconnection syndrome and indicate that he has acquired the capacity to cross-compare hemisphere information, at least with such alphanumeric characters. An examination of his data showed no indication that he was biased towards responding 'same' or 'different', and his errors were equally distributed between the two types of responses. There was no indication either that he performed better or faster with low-value than with high-value digits, and his results cannot be accounted for by some counting strategy.

Task 2

While the previous task required an exact mapping of bilateral information for correct comparison on the basis of identity, this task was designed to induce comparison in terms of less specific information, namely the binary category (odd-even) to which the digits belong. From the initial visual information, the identity of the 2 digits had to be accessed within each hemisphere so as to derive their respective category on which the comparison was to be performed between the hemispheres. The same digits as before were presented bilaterally, but the subjects now had to decide whether the 2 digits were of the same category (both odd or both even) or were of a different category (1 odd, 1 even). Although preliminary experiments indicated that A.A. was unable to make an odd-even decision in his right hemisphere, he was nonetheless tested on this task. If he were to be successful, it would indicate that the digit received by his right hemisphere could be transferred to the left hemisphere where it would be categorized and compared with the category of the other digit.

The procedure was the same as in Task 1 except for the following changes. Each of the 9 pairs of 'same' digits was used only once, which resulted, with the 36 'different' digit pairs, in a total of 45 stimuli which were presented twice in an experimental session. Two sessions were conducted, one with each hand. The task was to press one key if the 2 digits belonged to different categories and to press the other key if they belonged to the same category. There were 20 'different' category pairs (e.g., 2–7) and 25 'same' category pairs (e.g., 3–7), 9 of which were also of the same identity (e.g., 7–7).

Results The results are shown in the right of Table 1, as a function of the responding hand. A.A. responded at chance in all conditions (χ^2 (1) = 1.12 and 0.4, $P > 0.2$, for the left and right hand, respectively). Both L.B. and N.G. performed the comparison significantly above chance (L.B.: χ^2 (1) > 40, $P < 0.001$; N.G. χ^2 (1) = 10 and 19.6, $P < 0.02$, for the left and right hands, respectively). An examination of the data indicated that errors were equally distributed between 'same' and 'different' responses. N.G. was never able to give any information about the left digit, while L.B. could do so after some delay, and more quickly if the digits were identical than if they were not.

The main finding of this task was the capacity of N.G. to make a category comparison of the digits which she could not compare on the basis of their identity. Knowledge associated with the digits was thus amenable to a representation that could be made available across the hemispheres, at least in this type of binary categorization. It is also noteworthy that L.B.'s performance on this task was more accurate than in the preceding experiment, possibly suggesting a more efficient exchange of this binary information across the hemispheres in his case. On the other hand, A.A.'s failure to perform above chance confirmed that the information about the identity of the digit could not be transferred, and the inability of his right hemisphere to make an odd-even decision made the category comparison impossible to achieve.

Task 3

The evidence obtained in the preceding task suggests that cross-comparison can be achieved on the basis of categorical information, which raises the question of the nature of the information on which the decision is made. This task examined one aspect of this issue and sought to determine whether a unique abstract code was the actual basis for comparison.

The procedure was basically the same as before, except that a unilateral presentation condition was conducted first In this condition, the stimuli were the words 'odd' or 'even' and 1 of the 9 digits, presented one above the other in the left or the right visual field (*see* figs 2.1, 2.2). The stimuli were centred 7.5° from fixation, and the word was always above the digit. All combinations of digits and words in each visual field were used, resulting in a total of 36 stimuli, presented twice in an experimental session Two sessions were run, one with each hand responding. The subjects had to press one key if the digit and the word belonged to the same category and to press the other key if they did not.

In the bilateral task, the word and the digit appeared simultaneously in different visual fields (*see* figs 2.3, 2.4). Two experimental sessions were conducted and the instructions were the same as in the unilateral task. Because of his earlier performance, A.A was not tested on these tasks.

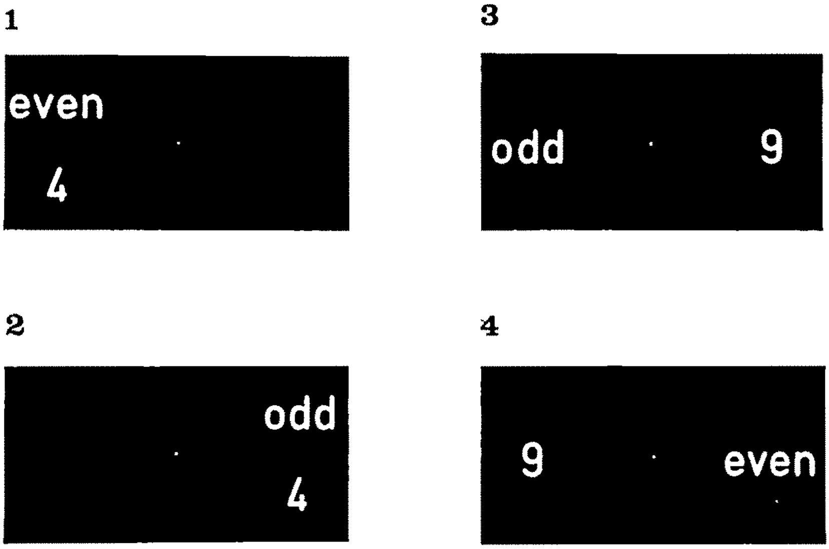

FIG. 2 Sample of the stimulus displays used in Task 3

Results. The results are shown in Table 2. In the *unilateral* task, L.B. performed significantly above chance in all combinations of visual field and responding hand (χ^2 (1) > 40, $P < 0.001$). Although he responded faster in the right than in the left visual field, this difference was not reliable ($F < 2$). N.G. performed significantly above chance in the right visual field with either hand (χ^2 (1) = 12.5 and 29.39, $P < 0.01$, for the left and right hand, respectively), but her accuracy was lower when responding to the

TABLE 2. CORRECT MEAN REACTION TIMES (RT) IN ms, SEM, AND PERCENTAGE OF CORRECT RESPONSES FOR L.B. AND N.G. IN TASK 3

		Left hand		*Right hand*	
		LVF[1]	*RVF*[1]	*LVF*[1]	*RVF*[1]
Unilateral condition					
L.B.	RT	1458	1421	1542	1349
	SEM	33.62	32.15	30.48	26.18
	% correct	88.89	91.67	90.27	97.22
N.G.	RT	1533	1488	1851	1481
	SEM	36.70	34.27	41.13	32.15
	% correct	65.28	70.83	58.33*	81.94
Bilateral condition					
L.B.	RT	1632	1553	1623	1471
	SEM	31.41	28.71	24.96	23.89
	% correct	76.40	79.16	69.45	78.78
N.G.	RT	1942	1919	1879	1757
	SEM	42.04	40.92	42.11	38.29
	% correct	47.22*	56.94*	41.67*	41.67*

[1] LVF and RVF refer to left and right visual field in the unilateral condition, and to the location of the word 'odd' or 'even' in the bilateral condition. * Not significantly different from chance.

left visual field: she performed slightly above chance with her left hand (χ^2 (1) = 6.72, $P < 0.05$) but not differently from chance with her right hand (χ^2 (1) = 1).

In the *bilateral* task (Table 2, lower part), L.B. was again capable of above-chance performance in all conditions. Response accuracy was the lowest when the word appeared in the left visual field and L.B. responded with his right hand (50/72, χ^2 (1) = 10.89, $P < 0.01$). He responded faster when the word and the digit appeared in the right and the left hemifield, respectively, than with the opposite arrangement, but this difference was only marginally significant ($P = 0.08$) His responses were both slower and less accurate in the bilateral than in the unilateral condition, and the difference was statistically reliable for both dependent variables ($P < 0.01$). N.G. responded at chance in all conditions (χ^2 (1) < 2, $P > 0.20$), even when the word and the digit appeared in the right and the left hemifield, respectively, a condition in which both hemispheres were capable of performing the required operations.

These results provide conflicting information regarding the capacity of commissurotomized patients to cross-compare a word and a digit in terms of the category to which each refers or belongs. While L.B. successfully performed this task, N.G. was not able to relate the 2 stimuli, and this cannot be attributed to an inability of either hemisphere to generate its respective information, at least in the condition where the word and the digit appeared in the right and the left field, respectively Instead, it suggests that the codes derived from each stimulus may not have been compatible to enable them to be related and compared across the hemispheres.

Task 4

The preceding tasks examined cross-comparison in terms of the identity or the category of the digits, irrespective of their numerical value The main function of digits, however, is to denote a quantity, and this experiment investigated the capacity of the patients to compare the digits in terms of their numerical value. Although the assignment of a given symbol to a given quantity is arbitrary, quantity itself is a fundamental concept and can readily be accessed from digits, and the comparison was made between digits considered as quantitative referents. The task involved basically the same design as before but, instead of making a 'same'-'different' judgment, the subjects had to decide which of the 2 digits was the higher. Such a task requires a comparison of the 2 digits projected to the two hemispheres in order to make a correct response.

The stimuli were the 36 'different' pairs of digits used in the earlier tasks. They were presented 4 times for a total of 144 trials. The task was to press the key on the side of the higher digit, and the subjects used the two hands to respond, pressing the right key if the digit on the right was higher than the digit on the left (e.g., 4−5) and vice versa (e.g , 5−4).

Results. The results are shown in Table 3, as a function of the side of the higher digit. All 3 patients performed the task at a high level of efficiency, and they responded significantly above chance (χ^2 (1) > 30, $P < 0.001$). They found the task very easy to perform and showed no hesitation in their responses,

TABLE 3 CORRECT MEAN REACTION TIMES (RT) IN ms, SEM, AND PERCENTAGE OF CORRECT RESPONSES FOR THE 3 SUBJECTS IN TASK 4

		Higher on the left	*Higher on the right*
A A	RT	1205	1167
	SEM	25 37	22 39
	% correct	83 33	88 89
L.B	RT	951	903
	SEM	15 72	14.82
	% correct	97.22	100
N G	RT	911	892
	SEM	19 54	17.42
	% correct	94 44	97 22

as indicated by relatively fast reaction times and small standard errors. Responses did not differ significantly as a function of the side of the higher digit.

When the patients were questioned about what they saw, neither A.A. nor N.G. provided any information about the left digit. When they first made a manual decision and then were questioned, they still had no idea about the identity of the left digit. For instance, A.A. was presented with the pair 5–7 and pressed the right key; when asked about the left digit, he said 'I guess it is smaller than 7, but that's all I know'. By contrast, L.B. was again able to tell the identity of the left digit, but with some hesitation and delay.

These findings illustrate the influence of informational demands on the capacity of commissurotomized patients to cross-compare digits. When they were directed toward processing the numerical value of the digits, rather than their identity, the information thus generated by one hemisphere could be made accessible to the other. However, the left hemisphere was unaware of the identity of the digit received by the right hemisphere, even though its quantitative referent must have been represented in some form. What is also remarkable about this performance is the high level of efficiency with which it was accomplished, suggesting that information about quantity or magnitude can be represented in a form more easily amenable to interhemispheric transfer than information about identity or category. In addition, there was no evidence of hemisphere asymmetry in this process, suggesting equally efficient transfer from either side.

Task 5

The results of the preceding task indicate that the patients were able to decide which digit was the higher and, therefore, were capable of detecting a difference between the digits presented in each hemisphere. This contrasts with a 'same'-'different' judgment between 2 digits (Task 1) which neither A.A. nor N.G. could perform and at which L.B.'s level of efficiency was much lower than it was in the preceding task. If the efficiency of interhemispheric comparison depends on the particular representation into which a given stimulus is coded, then, by inducing the patients into using numerical rather than identity information, they might succeed in accessing some difference between the digits and, therefore, in performing the 'same'-'different' judgment task. This task was designed to examine this possibility. However, not to make the task too difficult initially, this was attempted in two stages, described in this and the subsequent tasks.

The stimuli were the 36 'different' pairs of digits along with the 4 instances of the 9 'same' pairs, for a total of 72 stimuli which were presented once. One digit appeared in each visual field and the subject had to press the right key if the right digit was higher than the left, to press the left key if the left digit was higher than the right, and to raise both hands if both digits were equal (care was taken not to use the words 'same' and 'different' in the instructions).

Results. The results are shown in Table 4, as a function of the 'higher' or 'equal' response. All 3 patients performed significantly above chance in deciding which digit was higher and whether the 2 digits were equal (χ^2 (1) > 12, $P < 0.01$). There was a tendency for more errors when the subjects had to indicate

TABLE 4 CORRECT MEAN REACTION TIMES (RT) IN ms, SEM, AND PERCENTAGE OF CORRECT RESPONSES FOR THE 3 SUBJECTS IN TASK 5

		Higher	*Equal*
A.A.	RT	1277	
	SEM	21.14	
	% correct	79.17[1]	72.22
L.B.	RT	1007	
	SEM	14.51	
	% correct	97.22[1]	90.27
N.G.	RT	1078	
	SEM	17.42	
	% correct	93.05[1]	84.72

[1] Errors include pressing the wrong key and failing to respond.

that the 2 digits were 'equal' by raising both hands than when they had to press 1 of the 2 keys, but the difference was not significant in any of the subjects. The type of information that is generated from a given stimulus is therefore a critical factor in determining the efficiency of interhemispheric transfer. However, the design of this experiment was not exactly the same as the one used in the initial comparison task (Table 1).

Task 6

This task was in all points similar to Task 1, except for the instructions, in an attempt to examine whether the efficiency of interhemispheric comparison was a function of the information content on which the comparison was performed.

The same stimuli as in the preceding task were used. The only difference in procedure was the mode of response. The subjects responded with one hand, as in Task 1, and they pressed one key if 1 digit was higher than the other, irrespective of the side where it appeared, and they pressed the other key if the 2 digits were equal. Two experimental sessions were conducted, one with each hand.

Results. The results are shown in the left of Table 5, as a function of responding hand. A.A. proved unable to learn the requested assignment of the response keys during the practice trials, and he was not further tested. Both N.G. and L.B. performed the task at a high level of accuracy (χ^2 (1) > 40, $P < 0.001$), and they responded with no hesitation. An examination of the data showed no significant difference between 'same' and 'different' responses.

This task involved exactly the same design as Task 1 which N.G. performed at chance and in which L.B.'s level of accuracy was in the 70% range, and only the instructions were different. By orienting the patients to perform the task on the basis of the numerical value of the digits, it was possible to obtain a highly efficient comparison, suggesting that the informational demands of the task are a critical factor in determining the capacity of the patients to transfer information across the hemispheres. However, it is possible that the present finding reflected the practice and learning which the patients had acquired in the course of this series of experiments, and that they would have performed the comparison of Task 1 as efficiently had it been conducted at this stage of the investigation.

Task 7

This task was an exact replication of Task 1, and it was conducted about 1 h after Task 6 for N.G. and L.B. and after Task 5 for A.A. The interval was filled with an experiment on mental rotation. The main reason for this interval was to introduce this experiment to the subjects as a 'same'-'different' judgment task, and it was hoped that they would not resort to the earlier instructions.

TABLE 5 CORRECT MEAN REACTION TIMES (RT) IN ms, SEM, AND PERCENTAGE OF CORRECT RESPONSES FOR L.B., N.G. AND A.A. IN TASKS 6, 7

		Task 6		*Task 7*	
		Left hand	*Right hand*	*Left hand*	*Right hand*
A.A.	RT	–	–	1742	1601
	SEM	–	–	32.23	28.19
	% correct	–	–	47.22*	43.06*
L.B.	RT	1042	1092	945	908
	SEM	17.71	18.15	21.35	19.97
	% correct	94.44	86.11	63.89	73.61
N.G.	RT	1152	1215	1273	1194
	SEM	18.15	19.58	27.50	25.43
	% correct	93.5	88.89	54.17*	45.83*

* Not different from chance.

The procedure and stimuli were exactly the same as in Task 1, but only two experimental sessions were conducted. The subjects were told that this was a 'new' task, and they were instructed to press one key if the 2 digits were the same and to press the other key if they were different. No mention was made, as in Task 1, of the higher or equal value of the digits.

Results The results are shown in the right of Table 5, as a function of the responding hand. Both A.A. and N.G. responded at chance with either hand (χ^2 (1) < 2, $P > 0.10$). Although their reaction times were faster than in Task 1, suggesting an effect of practice, response accuracy was not better than in Task 1. L.B.'s performance was significantly above chance (χ^2 (1) $= 5.54$, $P < 0.05$ for the left hand, and χ^2 (1) $= 16.05$, $P < 0.01$ for the right hand) but at a level of accuracy more like that of Task 1 than of Task 6.

These findings suggest that the results of Task 6 (and of Task 5 for A.A.) were not simply due to practice or learning effects, by which the patients would have become capable of transferring information across the hemispheres during the course of this investigation. The results of the present task conform to those obtained earlier in Task 1 and indicate that interhemispheric transfer is a function of the informational demands of the task and therefore depends on the particular contents generated by the stimuli to be processed. While all 3 subjects could cross-compare digits at a high level of efficiency when they were induced into using the numerical value of the digits, only L.B. could do so, and with lower accuracy, when the instructions directed the subjects toward the identity of the digits.

Discussion

This series of experiments provides evidence that the capacity of commissurotomized patients to cross-compare visual information varies as a function of the information content that is derived from the stimuli. When the subjects were given no specific instructions except to make a 'same'-'different' judgment between the 2 digits (Tasks 1, 7), 2 failed the cross-comparison, presumably because they processed the digits in terms of their identity (either shape or name) which cannot be transferred in the absence of the corpus callosum. When the subjects were directed towards generating information about the digits, either in terms of a binary category (odd-even, Task 2) or of a numerical value (Tasks 4–6), interhemispheric transfer could be achieved, even when all characteristics of the task but the instructions were identical to the 'same'-'different' task (Task 1). However, Task 3 showed that the code into which the stimuli must be transferred is not a simple abstract code that can be derived from the word of the category and, even though L.B. could perform the comparison above chance in such a condition, the efficiency of interhemispheric transfer was significantly reduced.

It may appear puzzling that, in the absence of specific instructions, the subjects would process the digits in terms of their shape or identity rather than of their quantitative value in the 'same'-'different' judgment task Research on normal subjects, however, suggests that this is usually the case, as illustrated by the pattern of errors in such a task (Garner, 1974). For instance, there are typically more errors in response to visually similar pairs (e.g., 3–8) than in response to pairs of quantitatively close digits (e.g., 7–8). This does not mean that the quantitative value of the digits is not activated by their presentation, but it may not take precedence in performance. These considerations prompted further examination of L.B.'s data in the two 'same'-'different' tasks (1, 7), in which he was capable of above-chance performance. If the comparison was based on visual information, there would be a tendency for more errors with visually similar than dissimilar stimuli in 'different' responses. This was not the case, and the errors were randomly distributed across the digits, suggesting that the comparison was not based on visual information and that errors were due to inefficient transmission across the hemispheres. This aspect of the results will be further examined when addressing the question of individual differences among the patients in the General Discussion.

Part 2. Interhemispheric transfer of facial information

A second approach to the investigation of interhemispheric communication has relied on the capacity of the left hemisphere to provide verbal information about stimuli projected to the right hemisphere (e.g , Trevarthen and Sperry, 1973; Sperry *et al.*, 1979; Myers and Sperry, 1985). While such an approach typically examines information of which the left hemisphere is aware, a recent study by Gazzaniga *et al.* (1987) has indicated that verbal reports do not necessarily imply that the left hemisphere is aware of the identity of the information, but may in fact be aware of a difference between stimuli which is coded as a function of what the left hemisphere assumes this difference to be. This was the case when a binary discrimination was required, on the basis of the shape of 2 digits, confirming that pattern information cannot be coded in a form compatible with interhemispheric transfer.

This issue of the qualitative nature of the information being accessible across the disconnected hemispheres was further examined in the two subsequent experiments, using faces as stimuli Faces are particularly appropriate for examining the nature of interhemispheric transfer as they convey a variety of types of information about an individual and may thus provide indications about the characteristics of the informational contents that can be accessible across the hemispheres.

Task 8

The capacity of the left hemisphere to report on the emotional tone induced by the information presented to the right hemisphere was reported in the early stages of research on commissurotomized patients (Sperry, 1968; Gazzaniga, 1970), and was further investigated in the context of a study of self-recognition by the right hemisphere (Sperry *et al.*, 1979). The present experiment specifically examined the capacity of the two hemispheres to identify facial expression of emotion as well as to communicate between each other emotional information.

The greater capacity of the right than the left hemisphere to process facial expression of emotion is well established in normal (Bryden, 1982) as well as in brain-damaged patients (Dekosky *et al.*, 1980, Etcoff, 1984), but there is at present no systematic study on commissurotomized patients bearing on this issue. This was initially examined in the present experiment through unilateral presentation of faces expressing emotion (Ekman and Friesen, 1975). To ensure that the two hemispheres would be subjected to the same task demands, a pointing response was used, in which the patients had to match the emotion expressed by a face projected to one hemisphere with the emotion elicited by a specific scene.

The second part of the experiment also involved unilateral presentation of emotional faces but required a verbal response. One purpose was to examine interhemispheric transfer of emotional information. As a verbal response is emitted by the left hemisphere, the emotional information generated in the right hemisphere after left-field presentation will have to be accessed by the left hemisphere for the production of a response. A second purpose was to determine to what extent the right hemisphere advantage expected in the first part of the experiment when each hemisphere produced its own response would still be present when the response would be produced by the left hemisphere.

Several preliminary studies have been conducted to arrive at the present design (Sergent, unpublished data). The stimuli were 48 faces from the Ekman series of emotional faces (Ekman and Friesen, 1975). They consisted of 8 faces, 4 males and 4 females, each expressing 6 different basic emotions: happiness, sadness, anger, surprise, fear and disgust. The faces were mounted on slides and appeared either in the right or the left visual field, for a total of 96 stimuli. They were centred 6° from fixation and subtended a visual angle of about 3.5° in width and 4.5° in height. They were black and white photographs on a white background of 47.12 cd/m^2 and they were presented for 150 ms.

In the first part of the experiment, aimed at evaluating the respective capacity of each hemisphere to identify facial emotions, the subjects were presented with one face in one visual field and responded by pointing to 1 of 6 pictures located below the screen and each representing a scene assumed to elicit the type of emotion corresponding to the emotion expressed by the faces. Each scene depicted 1 of the 6 basic emotions: happy—wedding; sad—coffin; angry—someone stealing a purse; surprised—someone winning the lottery; fearful—someone pointing a gun at the viewer; disgusted—an open garbage bag with rats (scenes presenting a person did not show the face of the person). The capacity of the patients to associate each of these scenes with the 6 basic emotions had been established in earlier studies and was confirmed after the experiment. The subjects responded by moving either arm towards the selected picture. Twelve practice trials preceded the experimental session.

In the second part of the experiment, the same procedure for stimulus presentation was used, but the response was verbal without the pictures or any clue that could have been used by the left hemisphere. Although A.A. had participated in preliminary testing, he was not available for these experiments.

Results. The number of correct responses in the pointing task is presented in the top part of Table 6 as a function of visual field and type of emotion. Although they were not timed, the 2 patients responded quickly, without hesitation, and within about 2 s in most trials. Both patients performed significantly above chance in the two visual fields, and they were more accurate in responding to the happy faces than to any other emotional faces. Most of the errors resulted from confusions between the fearful and surprised faces and between the angry and the disgusted faces, as is also found in normal subjects (Ekman and Friesen, 1975). The 2 patients were more accurate in selecting the appropriate scene when the faces were presented in the left than in the right visual field. However, this difference was significant only in N.G. (34/48 and 22/48, respectively, χ^2 (1) $= 6.17$, $P < 0.05$) and failed to reach a reliable level of significance in L B. (30/48 and 21/48, respectively, χ^2 (1) $= 3.42$, $P > 0.05$).

The results of the verbal task are shown in the lower part of Table 6. They indicate that a verbal response, emitted by the left hemisphere, could be produced after presentation of an emotional face in the right as well as in the left visual field, and the pattern of response accuracy was essentially similar to that obtained in the pointing task. Accuracy was not significantly different in left and right visual field presentations for L.B. (28/48 and 26/48, respectively, χ^2 (1) < 1), but it was higher in left than right field presentations for N.G. (36/48 and 25/48, respectively, χ^2 (1) $= 4.55$, $P < 0.05$). Responses to right-field presentations were in nearly all cases brief and spontaneous, consisting of one word; responses to left-field presentations were not as spontaneous and often consisted in some paraphrase or in a description of a situation corresponding to the type of emotion expressed by the face. Although some of these descriptions were those of the scenes used in the pointing task, there were also situations from the personal experience of the subjects. Questions such as 'how did you feel then?' had to be asked to the patients to ascertain the type of emotion being aroused in such situations. Such questions never had to be asked after right-field presentations.

TABLE 6 NUMBER OF CORRECT RESPONSES[1] FOR L B AND N G IN IDENTIFYING EMOTIONAL FACES

	L B		*N.G.*	
	LVF	*RVF*	*LVF*	*RVF*
Pointing response				
Happiness	7	6	8	6
Sadness	5	2	7	3
Anger	3	3	5	2
Fear	6	3	5	5
Surprise	4	4	4	2
Disgust	5	3	5	4
Total (out of 48)	30	21	34	22
Verbal response				
Happiness	6	7	8	7
Sadness	6	4	8	4
Anger	4	3	6	3
Fear	5	3	5	4
Surprise	3	4	5	2
Disgust	4	5	4	5
Total (out of 48)	28	26	36	25

[1] No of faces for Task 8

A correlation analysis on accuracy data for each emotion type was conducted to examine the pattern of variations between and within visual fields and tasks. The coefficients are shown in Table 7 for the 2 subjects. Although the small number of conditions on which such coefficients were calculated precludes strong conclusions, they nonetheless indicate that there was no significant correlation between visual field within each task ($r\ (4) < 0.6$, $P > 0.10$, in both patients). In contrast, the patterns of response accuracy in the pointing and the verbal tasks were significantly correlated in right-field presentations for both patients ($r\ (4) > 0.85$, $P < 0.05$). In addition, performance in left-field presentations in the pointing and verbal tasks was significantly correlated in N.G. ($r\ (4) = 0.873$, $P < 0.05$) but not in L.B. ($r\ (4) = 0.701$, $P > 0.05$).

Comment. The results of the first part of this experiment confirmed a greater capacity of the right hemisphere to identify facial emotions, and this is the first demonstration in commissurotomized patients. However, this superiority of the right over the left hemisphere was only relative, as it did not reach significance in L.B., and as the left hemisphere performed significantly above chance in both patients. The main finding of this experiment, however, was the high degree of interhemispheric transfer, from right to left, of emotional information. The emotional content generated by the perception of expressive faces by the right hemisphere was almost entirely accessible to the left hemisphere, as suggested by the equivalent level of performance in left-field presentations whether the response was produced by the right hemisphere (pointing task) or was verbalized by the left hemisphere (verbal task). In addition, the superiority of the right hemisphere at identifying facial emotion was still prevalent, at least in N.G., when the response was made by the left hemisphere, further suggesting that it was on the emotional content generated by the right hemisphere that the left hemisphere based its responses in the verbal task. However, the nature of the verbal response was different for the two fields of presentation, and the fact that both patients had difficulty coming up with a concise response after left-field, compared with right-field, presentations suggests that the information transferred from the right to the left hemisphere was represented in a diffuse manner, accessible to awareness but not directly transposable into a verbal label. The patients could not readily qualify this information by a single word as they did after right-field stimulation, and they had to proceed by analogy and to be questioned to arrive at a precise response. This may indicate that what was transferred was more a general emotional feeling elicited by the expression of the face than a specific information about the nature of the emotion.

TABLE 7 CORRELATION COEFFICIENTS BETWEEN AND WITHIN VISUAL FIELD AND TASK IN THE FACIAL EXPRESSION TASK

		L.B.			
		Pointing		*Verbal*	
		LVF	*RVF*	*LVF*	*RVF*
Pointing	LVF	X	0.513	0.701	
	RVF		X		0.964*
Verbal	LVF			X	0.402
	RVF				X

		N.G.			
		Pointing		*Verbal*	
		LVF	*RVF*	*LVF*	*RVF*
Pointing	LVF	X	0.596	0.873*	
	RVF		X		0.877*
Verbal	LVF			X	0.416
	RVF				X

* Significant at $P < 0.05$

Task 9

Emotion is only one among the variety of items of information about a person conveyed by a face, and faces inform the viewer about many other characteristics such as age, gender, race, identity and related semantic attributes. Not all information is derived from the identity of the face, and emotion, as well as gender, age and race are contained in the visual facial configuration and can therefore be accessed in unfamiliar faces. Other information such as profession or achievements can be obtained only if stored knowledge about the individual has been activated. The purpose of this study was to examine the nature of the information which could be reported verbally after projection of familiar faces to the right hemisphere.

Pilot studies had been conducted on 2 previous occasions. A set of 20 faces known to both patients were used in the present experiments. The faces were those of politicians, actors, TV personalities and singers (*see* Appendix A). Each face subtended a visual angle of 3°–4.5° in width and appeared centred 6° from fixation. Each face was presented first in the left visual field and, for 10 faces, the next trial consisted in the presentation of the same face in the right visual field. For the other 10 faces, their presentation in the right field occurred later in the sequence. In addition, 10 faces unfamiliar to the subjects (French actors and politicians) were intermixed. The task of the subjects was to provide as much information as they could about the faces. However, pilot studies had indicated that both patients, and particularly N.G., had difficulty coming up on their own with information after left-field presentations, and a series of questions was asked, the order of which was adapted to the subjects' responses.

Results The main interest of this experiment lies in the verbal responses produced after presentation of a familiar face in the left field, and these responses lend themselves more easily to qualitative than to quantitative report. Quantitative data are presented in Table 8, as a function of type of information produced by the patients, and a sample of the responses of the 2 patients is presented in Appendix B.

Both patients were capable of providing verbal information about the faces projected to the right hemisphere, and their responses shared several common characteristics. In all trials where a face was projected to the right hemisphere, N.G. was never able to respond on her own, and she always had to be prompted. Without specific questions from the examiner, she remained mute, even when asked after the presentation nonspecific questions such as 'what did you see?' This was also observed in L.B., but only in half the trials However, on questioning, both patients came up with accurate information about the individuals, but this information was either categorical (man or woman, black or white, old or young) or semantic. The patients were always accurate at reporting the gender and the race of the face presented in the left field, but age estimation was made in terms of young, middle-aged or old, and, when asked to give a specific age, they responded at random within these categories (*see* Table 8). There was no significant

TABLE 8 NO OF CORRECT VERBAL RESPONSES TO FACES PROJECTED TO THE RIGHT AND LEFT HEMISPHERES AS A FUNCTION OF TYPE OF INFORMATION

		Gender	*Age** (+ or −)	*Race*	*Professional category*	*Achievement*	*Name*
L.B.							
Familiar	RH	20	22.5	19	16	13	3
	LH	20	8.6	19	18	18	18
Unfamiliar	RH	10	17.8	10	–	–	–
	LH	10	18.9	10	–	–	–
N.G.							
Familiar	RH	20	17.2	19	13	10	1
	LH	20	11.1	19	16	16	16
Unfamiliar	RH	10	15.0	10	–	–	–
	LH	10	19.2	10	–	–	–

* Expressed in mean difference from the actual age of the individual.

differences between left and right field in this respect, except for the age of familiar faces which was reported more accurately in the right than in the left field, presumably because the subjects knew the approximate age of the familiar individuals and were not relying only on visual information from the presented faces.

Neither patient was able to provide a verbal description of the visual characteristics of the face and, when asked whether they could 'picture' the face they were talking about, they responded negatively. For instance, when presented in the left field with a picture of the Ayatollah Khomeini, N.G. could tell that 'he was not an American', that he was 'not a nice man' and 'making war', but could not mention any characteristic about his face. The incapacity of the patients to access visual information about the face was evidenced by two other features of their responses. When a face that had first appeared in the left field was later presented in the right field, the patients always reported that this was the first presentation of this face In addition, when the same face was presented in succession in the left and then in the right field, the patients were never aware that it was the same face. For instance, after seeing the face of Richard Nixon in the left field and mentioning that he was a president, N.G. responded 'another President' to the presentation of Nixon's face in the right field. Thus interhemispheric comparison on the basis of facial identity could not be achieved, but it could be on the basis of semantic information about the individual.

Both patients could provide some information about the professional occupation and achievements of the individual, and they were also accurate in telling some characteristics of their personality. This information was, on two occasions, sufficient for L.B. to arrive at the right name. For instance, when presented with the face of Bill Cosby, L.B. could tell that he was a black actor, a funny one and very successful, and then said that it was either Eddy Murphy or Bill Cosby When asked why, he said that these were the only two black, funny actors that came to mind. When asked to guess, he responded 'Bill Cosby'. N.G. never succeeded in inferring the name of the face from the semantic information she could access. There was, however, one striking exception, in both patients It occurred with the presentation of the face of President Kennedy in the left field. The 2 patients were able to come up with his name, L.B. within 2 s, and N.G. after some questioning by the examiner.

Apart from the forementioned exceptions, neither patient could access the identity of the individual and, therefore, provide the name. There was no indication that they were even close to accessing phonemic information about the name. When questioned about the structure of the name, such as initial letter, number of syllables, similar names that came to mind, they had no idea regarding this information, and there was therefore no instance of the tip-of-the-tongue phenomenon (Brown and McNeill, 1966; Goodglass *et al.*, 1976). This is in contrast to responses to right-field presentations in which both L B. (on 3 occasions) and N.G. (on 1 occasion) initially experienced such a tip-of-the-tongue phenomenon and eventually came up with the correct name. In such conditions, they had access to some phonemic attributes of the word and could tell words that sounded alike. However, after left-field presentation, L.B. could on 4 occasions report the initial letter of the category name, such as 'P . . .' before reporting the word 'president', but this was not observed for the individual's name.

When familiar faces were presented in the right field, 18 were identified by L.B., and 16 by N.G. Only Michael Jackson was not recognized by either patient, and his face was also judged as nonfamiliar when presented in the left field Of significance is the finding that the other 3 faces which N.G. could not recognize in the right field (Frank Sinatra, Bob Parker and President Ford) had produced correct semantic information when presented in the left field. No unfamiliar face was judged as familiar in either field.

Comment. In both patients, information generated about faces perceived by the right hemisphere was accessible to the left hemisphere for verbal report. However, this information was not directly available for verbal responses, especially in N.G. and, without specific questions, she would have been unable to provide any information about the faces presented in the left field. This was not as striking in L.B who could come up with verbal responses without prompting on half the trials. It is noteworthy that this behaviour was not observed in Task 8 when the patients reported verbally on the emotion of the faces without prompting, which may indicate that emotional information to which the left hemisphere had access from the right hemisphere could be more easily translated into words than the type of semantic information generated in the present experiment.

While both patients reported verbal information about semantic attributes of the faces and of the individuals, two types of information were not accessible to the left hemisphere, namely the visual descriptions of the faces and their actual identity The absence of visual information in the left hemisphere about the face projected to the right hemisphere concurs with the main symptom of the disconnection syndrome. This was the case with such salient features of faces as the Ayatollah's beard or Robert Redford's blond hair even when the patients were able to provide semantic information that unmistakably corresponded to the individuals (*see* Appendix B). In addition, when the presentation of a face in the left field was immediately followed by the presentation of the same face in the right field, neither N.G. nor L.B. realized that they were the same face, but they could match them at a semantic level. It thus seems that a visual representation of the face was not transferred, and the semantic information about the face (e.g., age, gender) and about the individual (occupation, achievements) reported by the left hemisphere was derived from the outcome of processing taking place in the right hemisphere.

This dissociation between visual and semantic information may indicate that the patients were not resorting to cross-cueing strategies to obtain the information from the right hemisphere If cross-cueing had been involved, it should have applied equally to visual and semantic information and the left hemisphere should have been cued as easily, for instance, about the colour of Robert Redford's hair as about his gender. Instead, neither patient could report on the visual features of the faces, even when given a choice between two alternatives. It is noteworthy, in this respect, that the patients were very reluctant to venture a guess in response to a question, and they often responded that they did not know. The capacity to provide semantic information about a person requires that the face be individualized and recognized as that of a familiar individual, yet the identity of the face was not accessible to the left hemisphere. In as much as identity is best described by a name, it would be impossible for the right hemisphere to generate the verbal information necessary for the production of the appropriate name, and there was no indication that the left hemisphere had the slightest clue about the phonemic characteristics of the name. When L.B. succeeded in providing the name of the individual, this was done by his left hemisphere inferring the identity on the basis of the semantic information that could be accessed from the right hemisphere along with the knowledge held by his left hemisphere about the individual. This, however, was possible when there were only a few options. The capacity of both patients to give the name of President Kennedy after presentation of his face in the left field was exceptional and probably reflects the special status of this individual in everyone's memory.

GENERAL DISCUSSION

This study was designed to investigate interhemispheric transfer in the commissurotomized brain as a function of the information content underlying the performance of various tasks requiring the conjoint involvement of the two cerebral hemispheres. While the results confirm serious limitations in the exchange of information between the two disconnected hemispheres, they also provide evidence that the commissurotomized brain is functionally organized to subserve interhemispheric communication of some information content initially segregated between the two hemispheres and that such a communication is mediated by subcortical structures. These results confirm the functional capacity of the brainstem to subserve interhemispheric communication as demonstrated in patients with callosal agenesis (Jeeves, 1965), offer additional evidence of the selectivity of the disconnection syndrome following forebrain commissurotomy (Sergent, 1987), provide new evidence regarding the nature of the information that can be communicated between the two disconnected hemispheres, and may serve as a basis to infer some properties of the functional organization of cerebral processing.

The disconnection syndrome

The traditional view of the disconnection syndrome holds that the disconnected hemispheres are cut off from one another and constitute two independent cognitive processors running in parallel in the same brain (Sperry *et al.*, 1969; Bogen, 1985). This view has recently been qualified after some evidence has been obtained for interhemispheric transfer and it is currently suggested that 'there is general agreement that only rudimentary visual information is transferred between the hemispheres of a split-brain patient' and that 'the nature of the information transmitted in this manner is relatively limited in detail' (Kutas *et al.*, 1988, p. 571). This view was confirmed in the present study when only those tasks were considered that involved the same demands as those prevailing in the studies on which the traditional view of the disconnection syndrome is based. Thus the patients (at least A.A. and N.G.) were unable to make a 'same'-'different' judgment between 2 digits projected simultaneously, 1 to each hemisphere (Tasks 1, 7), and could not verbally report the identity of a stimulus presented in the left visual field (all tasks), nor could L.B. and N.G. describe the visual features of faces projected to the right hemisphere (Task 9). These findings are typical manifestations of the disconnection syndrome. However, they do not exhaust the variety of information generated in the processing of a given visual stimulus, and there is more to a visual object than its shape, identity and name. Other information, of a more qualitative and semantic nature, can be derived from the presentation of an object and is part of the knowledge that defines it. When the patients were induced into focusing on and processing this type of information, they were capable of efficient interhemispheric communication. This was best illustrated by the capacity of A.A and N.G. to compare 2 digits (Tasks 4–6) when they were directed towards using the numerical values of these digits, while they were unable to perform a 'same'-'different' judgment between the same pairs of digits (Tasks 1, 7) despite exactly identical experimental conditions. It thus appears that the disconnection syndrome following forebrain commissurotomy is specific to particular categories of information content and may not extend to all levels of information.

Before examining in more detail the characteristics of the information content and its influence on the efficiency of interhemispheric transfer, it is necessary to consider the differences in performance among patients in the various tasks carried out in this study.

Individual differences

Except in Tasks 4 and 5 where the 3 patients performed above chance, there were striking differences in their capacity to achieve interhemispheric transfer. In particular, L.B. performed above chance even in the 'same'-'different' task (Tasks 1, 7), which is at variance with predictions of the disconnection syndrome, as well as with his earlier performance on similar tasks (e.g., Johnson, 1984).

At least 3 factors can be invoked to account for L.B.'s unusual capacity: (1) inadvertently spared splenial fibres; (2) structural reorganization due to his relative young age (13) at operation; and (3) elaborate cross-cueing strategies (e.g., Gazzaniga and LeDoux, 1978).

The 2 MRI scans of L.B.'s brain have shown filmy substances in the centre of his splenium and, although the radiologist interprets the scans as indicating complete section of forebrain commissures, the possibility of some spared splenial fibres must be entertained, especially in view of L.B.'s unusual capacity. It could thus be that some splenial fibres subserve interhemispheric transfer in L.B., but several aspects of his results are not easily accounted for by such an explanation. For instance, L.B. is slow at reporting verbally information projected to his right hemisphere, and he performs the manual 'same'-'different' task several order of magnitude faster than he reports the identity of the left digit verbally. If visual information were transferred through the splenium, there should be very little difference in response time between a manual and a verbal response. In addition, L.B. was unable to describe the visual features of the faces projected to his right hemisphere while he could provide various items of semantic information about the faces. If interhemispheric transfer were mediated by spared splenial fibres, it would favour visual rather than semantic information, as recent evidence (Degos *et al.*, 1987) indicates that the splenium links occipital and posterior parietal lobes, but not the temporal lobes. Neither the occipital nor the parietal lobes appear to be involved in the generation of semantic information derived from faces, while the right temporal lobe is (Damasio, 1985), and the splenium cannot therefore subserve the transfer of semantic information which must reach the left hemisphere through another route. Results from simple reaction time tasks carried out on L.B. (Sergent and Myers, 1985) also cast doubt on a splenial route for interhemispheric transfer in his case. Such tasks provide an opportunity to estimate the time for unpatterned information to be transmitted between the hemispheres by the callosum. In neurologically intact subjects, the interhemispheric transmission time is of the order of 2 to 4 ms, as estimated by behavioural (e.g., Bashore, 1981) and physiological (Rugg *et al.*, 1985) measures. By contrast, transmission is estimated at 30 ms in L.B. (Sergent and Myers, 1985), which is not consistent with callosal transfer. It thus seems reasonable to assume that, if unpatterned visual information cannot be transferred through L.B.'s splenium, patterned visual information is even less likely to be transmitted by this route. Although some doubt may persist about the completeness of L.B.'s commissurotomy, this pattern of results is not consistent with interhemispheric transfer through possibly spared splenial fibres.

A second possible account of L.B.'s unusual performance would be that some structural reorganization of his brain has taken place due to the relatively young age at which his operation was performed. Two reasons seem to argue against such an explanation. One is that, even if L.B. underwent commissurotomy at a relatively young age, he was nonetheless already a teenager and cerebral plasticity may be quite limited by then (Jeannerod and Hécaen, 1979). Secondly, A.A. was only 1 yr older than L.B. at the time of surgery and yet, 23 yrs later, he is still unable to perform the 'same'-'different' judgment task. It is therefore unlikely that L.B.'s relatively young age at operation can account for his unusual performance.

A third possible explanation is the capacity of L.B. to use elaborate cross-cueing strategies (*see* Gazzaniga and LeDoux, 1978), by which one hemisphere emits some somatic signals picked up by the other. As pointed out by Sergent (1987), such a strategy cannot account for performance in a reaction time task. In addition, an analysis of L.B.'s data in Task 1 indicated no obvious pattern in his reaction times that could have suggested that he was using a counting strategy to compare the digits. Moreover, even in an untimed task such as Task 9, where L.B. could have resorted to a cross-cueing strategy, the dissociation in the capacity to transfer visual and semantic information argues against the recourse to this strategy which should have been equally applicable to both types of information.

Although none of these explanations can entirely be excluded, they seem to be insufficient to account for the whole pattern of results displayed by L.B. in this study. A different type of interpretation may then be suggested on the basis of the evidence gathered in this study, indicating a dependence of interhemispheric transfer on the nature of information being processed. As noted in the Introduction, a given stimulus may activate a variety of types of information representing different levels of knowledge. It is conceivable that, when L.B. processes a digit in terms of its shape, as required in Task 1, information associated with the numerical value of the digit is also activated, albeit not as strongly, as such information proves to be readily communicable across the hemispheres (Tasks 4–6). It may thus be that L.B.'s performance in the 'same'-'different' judgment task was based on processing some weakly activated information about the numerical value of the digits, even if the main focus of attention was on the shape of the digits. In support of this interpretation is the analysis of the errors in Tasks 1 and 7, which indicated that digits were not compared on the basis of their visual shape. This interpretation would suggest that L.B. is simultaneously activating different categories of information about a stimulus among which some are represented in a form compatible with subcortically-mediated interhemispheric communication.

One obvious question is why L.B. alone would be capable of using such multilevel representations of knowledge about a given stimulus efficiently. Although there is no unequivocal answer, it may be suggested that some idiosyncratic characteristics of L.B. allow him a greater processing efficiency. His intellectual and cognitive abilities are remarkably high among commissurotomized patients, which must have some neural correlates possibly expressed in terms of efficient synaptic connections that determine the organization of knowledge. In addition, L.B., who considers himself as a 'professional subject', devotes much time and thinking to finding ways of overcoming difficult problems to which he is exposed and, in doing so, keeps his mind alert and open to any possible associations between items of information of which he may think. Neither A.A. nor N.G. adopt such an attitude towards testing and, although serious and able to concentrate, they are not readily concerned with finding alternative ways of solving a task which they initially fail. It may thus be that L.B. has developed the capacity to encode information at different levels at once more efficiently than other patients, and that his unusual performance in the 'same'-'different' task reflects the availability of several codes on which to carry out a given task, thus enhancing the probability that some of these codes may be appropriate for interhemispheric communication. Although admittedly speculative, but open to investigation, this interpretation may be consistent with the evidence that the efficiency of interhemispheric comparison depends on the 'state of knowledge' of the hemispheres at a given time.

In spite of striking differences among subjects in the levels of performance they achieved across experiments, it is noteworthy that their performance varied essentially in parallel. Success in a task by A.A. and N.G. (Tasks 4, 5) corresponded to an almost perfect performance by L.B.; success by N.G., but not by A.A. was paralleled by a high performance by L.B.; failure by both A.A. and N.G. was associated with only slightly above chance performance by L.B. There is therefore some consistency in the performance of the subjects across tasks, and the issue of individual differences can be left aside to focus on the nature of information content underlying interhemispheric communication.

The nature of the information content

One critical assumption made in the design of this study was that the same stimulus potentially conveys different levels or categories of information, and that these different levels are not equally accessible and entail different representations. The results of this study validate this assumption and indicate that these representations also differ in their communicability from one hemisphere to the other. It is necessary to examine what differentiates them.

The present results as well as earlier findings suggestive of the disconnection syndrome were typically obtained when either the shape or the identity of a given stimulus was the information of relevance in the comparison. Current evidence of interhemispheric disconnection is therefore limited to visual and identity information. In the visual domain, the considerable cortical space devoted to the reconstruction and elaboration of the attributes of the stimulus (e.g., Van Essen, 1985) prevents the relatively limited subcortical space from transmitting such a large amount of information at once. The large number of splenial fibres is normally required for such a transmission which cannot therefore be taken over by other commissures (Sidtis *et al.*, 1981). Also uncommunicable between the hemispheres was the information about stimulus identity, which is the end product of semantic processing of the initial shape but which does not carry by itself any information about the referent.

It may then be of interest to note that the two types of information resilient to interhemispheric transfer in the commissurotomized brain do not carry by themselves much relevant knowledge about the stimulus. A shape is of no use for guiding behaviour if it has no meaning associated with it, and an arbitrary symbol or label assigned to a shape such as a digit or a face is of little if any practical value if no other information is accessed. These two types of information stand at the two ends of cognitive operations on a given stimulus, and it is the processes performed in between and the information that they generate that provide the relevant knowledge about the stimulus which can be used to guide behaviour. One central finding of this study then is that it is specifically the information carrying the relevant knowledge about a stimulus and necessary for guiding behaviour which is communicable between the hemispheres in the commissurotomized brain. This was the case for category and numerical value of digits (Tasks 2, 4–6), the emotional, visual semantic (e.g., gender, race) and personal semantic (e.g., occupation, nationality) information about faces and individuals (Tasks 8, 9). It is noteworthy that, except for orientational and emotional information, the subjects had to be directed towards processing specific categories of information about a stimulus to achieve interhemispheric transfer (but *see above* with respect to L.B.). Interhemispheric communication was thus a function of the state of knowledge of the brain at a given time and, therefore, of the particular pattern of activated information about the stimulus.

Role of subcortical structures

Interhemispheric transfer in the absence of forebrain commissures, and in conditions that control for eye movements and cross-cueing, can be achieved only through the mediation of subcortical structures. Evidence had already been reported for the role of these structures in the transfer of rudimentary information (Trevarthen and Sperry, 1973; Holtzman, 1984), and research in patients with callosal agenesis has shown the critical importance of the midbrain in interhemispheric integration while at the same time indicating definite limitations in this function, particularly with respect to the transmission of fine-grain sensory information. The present results suggest that, in commissurotomized subjects, what is being communicated between the hemispheres goes far beyond rudimentary visual information.

The notion of interhemispheric transfer typically used in recent studies carries with it the implication that subcortical structures serve only as pathways that link the two hemispheres. Yet subcortical structures contain very few commissures (the collicular and posterior commissures) while they comprise a large variety of processing stations intricately connected between each other and with cortical structures (Tigges and Tigges, 1985). This suggests that hemispheric information may not cross the midline without being processed at such a level, as it does through the corpus callosum. Instead, subcortical structures may be involved in the treatment of this information and send back to both hemispheres the outcome of their operations. In this sense, subcortical structures are an integral part of the cerebral network and are in a central location to coordinate and integrate the activity of the two hemispheres. It is, in fact, even doubtful that 'rudimentary visual information' as such is being *transferred* by subcortical structures. For instance, it has been shown that commissurotomized patients cannot tell the orientation of an arrow appearing in the left field (Holtzman, 1984; Sergent, 1987) but can use this orientational information for processing stimuli appearing in the right field. While this suggests that some information about the orientation of the arrow was represented in the left hemisphere, there is no guarantee that it was in a visual form. If visual information was transferred as such, it would imply that each disconnected hemisphere can represent visual information about the ipsilateral field, in addition to the highly elaborated information about the contralateral field. Current evidence about the organization of the visual cortex (Van Essen, 1985) offers no indication that such information, conserving the retinotopic arrangement of the ipsilateral field, can be represented in cortical areas. It thus seems that visual information received by the two disconnected hemispheres is combined and integrated at a subcortical level from which some abstract of the visual scene is sent back to the two hemispheres.

While subcortical mediation of emotional and orientational information is well established (e.g., Tucker, 1981; Tigges and Tigges, 1985), a similar involvement of subcortical structures in the processing of higher-order cognitive information is not as well documented. There is some evidence for such an involvement (e.g., Fessard, 1954; Penfield and Roberts, 1959; Brown, 1977; Castaigne *et al.*, 1981), but the actual structures and mechanisms underlying their contribution to cognitive functions still elude us. There are, however, some indications that the outcome of operations carried out in one hemisphere are available to subcortical structures which then activate both disconnected hemispheres. For instance, Gazzaniga (1972) reported findings from an event-related potential study in which response to a stimulus presented unilaterally generated the same pattern of brain potential in the two hemispheres of commissurotomized patients. More recently, Kutas *et al.* (1988) observed that completion of a sentence by a semantically anomalous word presented in the right visual field generated a specific N400 wave over both the left and the right hemispheres. Of particular relevance was the *simultaneous* generation of this brain potential over the two hemispheres, suggesting that operations initially performed in the left hemisphere must have been related to subcortical structures from which both hemispheres were activated in synchrony. While these findings were obtained in conditions differing from those prevailing in the present study, they nonetheless indicate that a common subcortical source was responsible for a specific bilateral hemisphere activation as a function of the semantic nature of the information received by one hemisphere. No such pattern of bilateral activation seems to be generated in response to the processing of visual information or the identity of a stimulus (e.g., Gevins, 1983), but more research would be required to examine this question systematically.

It may thus be suggested that subcortical structures participate in the processing and derivation of semantic information, and they are activated in such a way that they contribute to keeping the two hemispheres informed of their respective informational contents. However, the information thus accessible across the two hemispheres is not highly specific. As suggested by the evidence reported by Kutas *et al.* (1988), and consistent with the behavioural responses obtained in the present study, one hemisphere may have access to the semantic information derived from a stimulus received by the other hemisphere but not to the identity of this stimulus. The semantic information that can be communicated in this manner may then be sufficient to determine the production of unified behaviour, as long as alternative choices are limited. In this sense, subcortical structures do not act as commissures, but as integrative processors that keep the two hemispheres in phase and provide them with a common context within which to perform their respective operations. It is noteworthy that the bilateral activation observed by Kutas *et al.* (1988) in the commissurotomized brain was qualitatively similar to the pattern of activation observed in neurologically intact subjects, suggesting that this integrative function of subcortical structures may not be specific to the commissurotomized brain but characterizes normal cerebral functioning.

The limitation of subcortical structures for a complete integration of information held in the hemispheres is evidenced by the incapacity of each hemisphere to access the full contents held by the other hemisphere, which is a direct consequence of the callosal disconnection. When interhemispheric communication is examined as a function of visual or identity information, the typical disconnection syndrome prevails. By contrast, when it is examined as a function of less specific information, communication may be achieved, especially when the information does not have to be translated into verbal codes. Yet each hemisphere remains unaware of the nature of the information received by the other, but two points may be noted in this respect. One is that unawareness of the information concerns the *identity* of the stimulus. For instance, when the patients were successfully comparing digits as a function of their numerical value, the only way of describing this value was by referring to the *name* of the digit, which could not be accessed, but this does not imply that some abstract information about the magnitude of the digit, not verbalizable otherwise than by the name of the digit, was not represented in the other hemisphere. The second point is that unawareness does not mean absence of content, as clearly illustrated by the high efficiency with which performance was achieved in some of the tasks. The behaviour of N.G. when presented with a familiar face in her left visual field is of interest in this respect. She was not able to provide a verbal response on her own, yet her left hemisphere contained information about the face and the individual, but not at a sufficiently activated level to reach awareness. The verbal questions may then have served to increase the level of activation of this information which could then achieve full awareness. The fact that some questions were not followed by verbal responses while others were suggests that it was not the right hemisphere which was understanding the question and sending the appropriate information accordingly; instead, it reflects the selectivity of subcortical communication.

The view that subcortical structures play a unifying and integrative role in cerebral functioning (e.g., Fessard, 1975; Penfield and Roberts, 1959; Jeeves, 1965) may have fallen into oblivion after the dramatic discovery of the disconnection syndrome resulting from forebrain commissurotomy. While the focus of investigation and interest then shifted to cortical processing and callosal transmission, the evidence that the commissurotomized brain is capable of unified behaviour strongly suggests that the corpus callosum does not have exclusivity of interhemispheric communication. Subcortical structures allow the two hemispheres to share some of their respective information content, and the information thus shared carries much of the knowledge relevant and necessary for adaptive behaviour. In so far as the brain is designed for the regulation of overt behaviour and the production of adaptive responses to stimulation from the environment, subcortical structures appear to be essential for the coordination and integration of hemisphere activity.

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APPENDIX

A *List of faces used in Task 9*

Presidents Kennedy, Nixon, Ford, Carter, Reagan; Ted Kennedy, Robert Redford, Burt Reynolds, Jackie Kennedy, Bill Cosby; Farah Fawcett, Bob Parker, Queen Elizabeth, Prince Charles, Ayatollah, Brejnev, Elvis Presley, Michael Jackson, Jesse Jackson, Frank Sinatra.

B *Sample of verbal responses to familiar faces in Task 9* (irrelevant comments and jokes by the patients have been left out)

N.G., left visual field. Robert Redford

(Question) Is this a man or a woman? (Answer) *I am not sure . . . a man* How old? *Young, quite young* How young? *25 or 30.* Does this man look familiar to you? *I think so.* Where have you seen him? *I don't know. What do you think?* What does he do for living? *Is this a movie actor? . Yes, an actor.* What kind of character does he play? *. . A playboy.* What do you mean? *He is quite handsome, don't you think?* How do you know he is handsome? *Well, I know* Can you picture him? Can you describe his face? *No, I can't say what he looks like but I think he is handsome* What is the colour of his hair? *I don't know* Rather blond or dark? *I don't know, maybe dark. I prefer men with dark hair What do you think?* Would you invite him to your place? *Of course . But . . I don't know about B . What am I saying?* Can you recall some movies of his you saw? *I know I have seen him play, but I can't tell any movie* Do you know his name? *No* Do you have any idea? *No* Is it a long or a short name? *I don't know.* Do you know by what letter it starts, or what it sounds like? *No idea.*

N.G , right visual field Robert Redford

Another actor. Did I show this face before? *No* Does he look like the one you just saw? *No I don't know* Do you know who he is? *Yes He is quite handsome too . . Robert Redford.*

N G., left visual field Ayatollah

Is this a man or a woman? *He is quite a man.* What do you mean by quite a man *I don't know . I don't like him.* Why? *I don't know he does not feel right.* How old is he? *Quite old* Can you describe his face? *An old face.* Anything in particular? *No.* What does he do for living? *Bad things . . What am I saying? I don't know . He is not a nice man.* What do you mean? *I don't know . . just an impression.* What did he do? *Did he harm people?* . Where does he live? *I don't know.* Does he live in the US? *No He is not American. . . He lives far . it's not here* Do you know where? *No He is making war.* Do you know his name? *No* Is it a long or a short name? *I have no idea* More than 2 syllables? *I don't know*

N G , right visual field: Ayatollah
Ali baba Who is that? *You know, the old priest We often see his picture.* Did I show you this picture before? *No, I would not forget this face.*

N.G , left visual field· Richard Nixon
Is this a man or a woman? *A man* How old is he? *Middle aged.* Could you be more specific? *About 35, 40.* What does he do for living? *He plays games* What do you mean? *I saw him on TV.* Does he present game shows? *No. I don't think so* What was he doing? *I don't know. I don't think this is an actor Is he a politician? . . Yes, a politician.* What kind of politician? *I don't know. What do you think?* I don't know, you tell me *He is powerful . . . Is he a president?* Well, you tell me. *I think he is a president.* Is he a president now? *I don't know Is this Reagan?* What do you think? *I don't know, that's a president, but I can't tell which one.*

N G , right visual field, Richard Nixon
Another president Is this the president I just showed you? *No I don't know . . I don't think so* Who is he? *Tricky Dick.* Who is that? *You don't know Tricky Dick? That's Nixon*

N G., left visual field· President J F Kennedy
Is this a man or a woman? *A man* How old is he? *Rather young* How old? *35* Do you know him? *Yes, I know who this is, I like him.* What does he do? *That's President Kennedy.*

N G , left visual field Queen Elizabeth (wearing a crown)
Is this a man or a woman? *A woman* How old is she? *Old* How old? *70.* Do you know this person? *Yes, I think I know* Where did you see her? *TV, newspaper* What does she do for a living? *Nothing* What do you mean? *I don't know What do you think? Is there something wrong with me?* What's her occupation? *I don't know* Is she an actor, a politician? *I don't know* Does she work? *No.* Is she poor? *No. . . She gets money from people* You mean she is a beggar? *No She is rich* How does she do that? *I don't know . Do you know?* Do you know who she is? *No.* Do you know where she lives? *I don't know.* Is she American? *No* From which country? *I don't know*

N G , right visual field, Queen Elizabeth
That's the Queen Have you seen her face earlier? *No. I would not have missed it*

L B., left visual field, Richard Nixon
That's a man. How old is he? *I'd say in his thirties* What race? *White.* Any particular characteristic about his face? *None that I can think of.* What's the colour of his hair? *I can't tell.* Light or dark? *In between* Is it someone you know? *I think so . He is familiar* Where did you see him? *Well . I don't think I have ever met him. Must be on T V.* What kind of job does he do? *I guess he holds a public function* What do you mean? *To be on T V you must be a public figure* Could you be more specific. *Well Let me put it this way He is not a singer He is not an actor. He may be a news man . . but it does not seem right He is a politician Yes.* Would you see him in Sacramento or Washington? *Definitely in Washington. He is a federal politician* What position? *I think 'P' comes to mind . . President* Is he president now? *I am not sure. No Definitely not . . I said he was not an actor It's funny! I can tell who he is not but I can't figure out who he is.* Do you have any clue about his name? First letter? How many syllables? *No*

L B., right visual field Richard Nixon
President Nixon Is he the one you just saw? *I don't think so but he was a Republican too*

L.B., left visual field Ayatollah
A man, in his fifties Is it someone familiar? *Yes I feel I have seen him before* Can you describe his face? *Just an ordinary face* Is he black or white? *White* Any particular features? Was he wearing a hat in the picture? *No, none that I could tell* Does he have long hair or short hair? *Usual length, like mine* Have you ever met him? *No.* Where does he live? *Far. New York? No he lives abroad* What does he do? *Well. If he is not American and I know him, he must be a politician.* Do you know in which country? *I can't figure out Somehow, Gandhi comes to my mind . . . but I know it is not right. I have a weird feeling about him but I don't see why It's someone who has a lot of influence*
Any idea about his name? *Not the smallest clue*

L.B , right visual field, Ayatollah
Khomeini Did I show you this face before? *No. That's the first time I see it here*

L.B , left visual field. Bill Cosby
That's a man How old? *Rather young . in this thirties.* How does he look? *I can't tell . . . he is black.* What

does he do? *Don't know . . . but I think something funny.* What do you mean? *I have the impression that he makes people laugh.* How does he do that? *I think he is an actor Yes . . a comedian and a good one at that* Can you think of some of his movies? *No But he is quite successful.* Do you know his name? *No* Is it a long name or a short name? *No idea . It must be either Eddy Murphy or Bill Cosby That's the only black comic actors I know* You're right, its one of them. Could you guess which one? *Bill Cosby* Why? *I don't know You asked me to guess*

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BOOK REVIEWS

Clinical Neurology. By C D Marsden and T. J. Fowler. Physiological Principles in Medicine Series. 1989. Pp. 464. London: Edward Arnold. Price £19.95.

Clinical Neurology is the companion volume to *Neurophysiology* by R. H. S Carpenter in a series that is designed for students to link the understanding of normal function with disorders of various bodily systems. In this case, the transition is softened by the first 3 chapters which focus attention on 'the patient's complaints' and 'the doctor's examination'. The remainder of the text presents an orderly consideration of diseases of the nervous system with 9 chapters being contributed by other authors.

The opening chapter deals with the principles of localization and pathological diagnosis by clinical acumen, imaging techniques and other investigations. The illustrations of CT and MRI scans, accompanied by diagrams of the main anatomical features, will be a useful guide for students No formal instruction in history taking is provided but there is a pleasing chapter on symptoms and their interpretation. The approach to the patient with headache, facial pain, impaired consciousness and vision, giddiness, pain in the arm and leg, movement disorder and mental changes is described logically and succinctly. The view that 'epilepsy in the infant indicates some serious metabolic or structural cause' and that 'epilepsy beginning in the younger adult often is the first sign of a cerebral tumour' seems unduly pessimistic. Hyperventilation is mentioned under the heading of 'episodic loss of consciousness' but could well find a place in other sections such as vertigo because it so often mimics organic disease and passes unrecognized.

The outline of neurological examination follows conventional lines, mercifully making clear that every aspect does not have to be covered in every examination and that specific procedures will be determined by the history A practical Mini-Mental State Examination and a simplified description of aphasia is included, although the association of a nonfluent aphasia with good repetition of phrases suggests a transcortical aphasia rather than Broca's aphasia as appears in the table. Abnormalities disclosed by physical examination are analysed and well illustrated, concluding with an excellent section on coma and its management.

The different categories of neurological diseases are described systematically, but it was surprising to find no obvious link to the companion volume of this series. The coverage is comprehensive and is aided by tables, although the length of some could prove daunting to novices. More prominence could be given to the neurological presentation of AIDS in a subsequent edition since, as the authors note 'neurosyphilis, and now AIDS, are the great mimics of neurological disorders'. The final chapter covers common psychiatric syndromes and their links with organic disease in acute confusional syndromes and mental retardation.

As is customary in a student text, no references are cited to substantiate statements made and, for the most part, none is needed. A list of recommended reading and references, mostly of standard texts, is provided at the end of the book, but the distribution of subjects is uneven. For example, of 7 references cited to cover 4 topics, dementia, epilepsy, migraine and movement disorders, 3 are devoted to epilepsy and none to headache.

'Clinical Neurology' is well presented and printed with appropriate subheadings, many diagrams and clear photographs that make it a pleasure to read It should prove a useful addition to the reading list for senior medical students.

JAMES W. LANCE

Medical Neurology. By David Chadwick, Niall Cartlidge and David Bates. 1989 Pp. 506. Edinburgh: Churchill Livingstone. Price £24.95 (paperback).

Neurology and General Medicine. Edited by Michael J. Aminoff. 1989. Pp. 824. Edinburgh: Churchill Livingstone. Price £60.00.

On both sides of the Atlantic, general physicians with an active interest in clinical neurology and clinical neurologists who continue to practise general medicine are almost extinct. Rapid advances in knowledge, increasingly sophisticated diagnostic techniques and demandingly complex therapeutic strategies irrevocably determined the evolution of specialization. While the notion of a renaissance medical scholar equally competent in all branches of medicine has a certain romantic attraction and intellectual nobility, in the present world such a practitioner would be unsafe, if not frankly dangerous. It is also unlikely that he would be often available for consultation as he would be obliged to spend a certain amount of his professional time defending alleged negligence and malpractice in the courts of law.

This historical reality is associated with considerable educational problems. The young physician in training has to acquire reasonable competence and confidence in clinical neurology as he is certain to encounter such problems in the course of his everyday work; the trained clinical neurologist has to strive to keep up with advances in other medical specialities which may critically influence his own judgements and decisions. It is agreeable to be able to welcome two fine new books which specifically deal with these problems. *Medical Neurology* is aimed at the trainee physician. Crisp in style, profusely illustrated, including excellent line drawings, flow diagrams, comprehensive and comforting lists of causes, differential diagnoses and treatments, the text accurately focuses on the essential needs of a physician in training. The overall approach is that of conventional and well tried clinical method: symptoms and signs, symptom complexes and specific pathologies. These are clearly presented, each chapter ending with a few suggestions for further reading. This admirable guide for the neurologically perplexed young physician is warmly recommended.

Neurology and General Medicine aims to bridge the ever widening chasm between neurology and other medical specialities as encountered by the established clinical neurologist. The neurological features, manifestations, complications and sequelae of a very wide variety of general medical conditions are authoritatively presented by acknowledged experts. Thus, considering cardiovascular disorders alone, there are separate chapters on aortic disease and surgery, cardiac surgery, congenital heart disease, acquired heart disease, infective endocarditis, hypertension, hypotension, cardiac arrest and circulatory consequences of acute neurological lesions. Throughout the text each topic is considered in detail and depth and exacting editorial skills have produced an attractive and eminently readable text. Most of the chapters have well over a hundred references. The breadth of the book is most impressive and encompasses bedside clinical techniques as well as recent advances in the relevant basic sciences. For example, the neurologist who is not familiar with the technique of demonstrating asterixis in the lower limbs of a comatose patient will need to consult this book as well as those who would wish to admire spectacularly beautiful electron micrographs of the arachnoid in experimental meningitis.

These books are assured of an enthusiastic, appreciative and grateful readership.

GERALD STERN

Contemporary Issues in Neurological Surgery. Mild to Moderate Head Injury. By Julian T. Hoff, Thomas E Anderson and Theodore M Cole. 1989. Pp. 246 Oxford: Blackwell Scientific. Price £13.50.

Mild to Moderate Head Injury is a multiauthor work taken from a symposium on the subject held at the University of Michigan in 1987 organized by their professor of neurosurgery, Dr Hoff. There can be no doubt that the book addresses a most important subject, one which has been little studied since head injury research was put on a rational path by the introduction of the Glasgow Coma Scale in 1974 by Jennett and Teasdale. This path has led to a significant improvement in the understanding and management of severe head injuries which the scale defined. It is clear that the scale, for all its merit and with the repeated encouragement of its authors, needs redefining in its definition of mild (GCS 13 – 15) and moderate (GCS 9 – 12) injuries.

The book covers four main areas and ends with a fifth section of conclusions written by the chairman of the various topic workshops. The first group covers definitions, epidemiology and neuropathology, including neuropharmacology and evaluation of experimental models. This wide-ranging section sets out the scale of the problem: 1½ million patients per annum in the United States at a cost of $1.1 billion in 1981, and discusses its aetiology. The second and third sections deal with diagnosis and management, pointing the way to further improvements by organized trials and data collection. The fourth section addresses the enormous problem of rehabilitation and assessment of neuropsychological damage.

A book of this type is always a little uneven in its style and content, but in a new or developing field such as this, it brings out the controversial nature of its subject, not the least of which is in the definition of the subject itself. Here there is some disagreement on who are to be considered and how best to define them. The second section deals largely with this topic. The most contentious area is in the definition of the mild injury. Clearly there are those who consider the current definition of GCS 12 – 15 too wide, indeed it is obvious that there is a world of difference between a patient who has a seemingly trivial injury and is perfectly well apart from a headache, and one who lies asleep in bed, but on arousal releases a string of inappropriate abuse. Yet both can be classified by the scale as mild head injuries. No resolution to change the classification is made.

The section with the greatest potential clinical interest is that on management. Perhaps reflecting the paucity of new information, this has the least that is new, and there are no surprises for the neurosurgeon. Nevertheless, it would be helpful to know how Professor Miller, who takes the lion's share of this section and is an acknowledged expert and exponent of intracranial pressure monitoring, sees the field developing. For instance, it would be useful to know how the policy of scanning all head injuries regardless of severity as is adopted in some US centres influences outcome. Does it reduce mortality or does it lull the clinician into a false sense of security and mask the development of late haematomas? However, his description of management in the head injury unit set up by him and currently used in Edinburgh should serve as a model for management in the United Kingdom.

It is sad to reflect that the information contained in a book of this type will probably never reach the accident service junior staff and admitting surgical registrars who look after the bulk of mild and moderate head injuries in the UK. Lack of interest in this Cinderella subject, compounded by a shortage of funds for overall health care, will prevent substantial developments for many years.

On a point of accuracy, it will come as a surprise to Professor Teasdale to discover that he is the chairman of the department of neuropathology in Glasgow Whatever, this is an interesting and useful book which I would recommend to anyone involved in the management of head injuries or their sequelae.

MICHAEL POWELL

The Bridge between Neurology and Psychiatry. Edited by E. H Reynolds and M. R Trimble. 1989. Pp. 409. Edinburgh: Churchill Livingstone. Price £47.50

This volume of essays, originally planned from a mutual conviction that the divergence of neurology and psychiatry has gone too far, is also presented as a tribute to the late Sir Denis Hill. The title is taken from an address by Denis Hill in which he depicted the 'bridge' joining the two disciplines as constructed from the materials of common techniques, shared ideas, and an endeavour to solve the same problems; but essentially created from a common intellectual curiosity. His advice was sought when the book was first planned and two of the most salient chapters are from his pen.

I find it particularly fitting that on the eve of the 150th anniversary of King's College Hospital a tribute of this nature should be paid to one of the hospital's best teachers. Saturdays were for rugby, either at Dog Kennel Hill where Denis Thatcher used to referee on occasion, or at various sites south of the Thames.

Even so, Denis Hill filled the lecture theatre in the morning with an hour's lecture followed by a demonstration. Students were liable to be asked questions, but always with the recognizable aim of promoting a lively discussion. For Denis Hill teaching was an exercise of the intellect, manifested by the array of talent he attracted to his ward rounds and outpatients I found myself quoting one of his aphorisms, 30 years after the event, when preparing a paper on the neurology of familiarity. A certain quizzical humour often pervaded his teaching: few people ever understood why, in Richardson's *Textbook of Medicine* aimed at medical students, he should have chosen to present the whole realm of clinical psychiatry in terms of the id, ego and superego.

Lishman and Reynolds, in particular, acknowledge a historical schism: psychiatry emerging from philosophy and social attitudes and neurology from mainstream medicine. But many neurologists would term psychiatry as behavioural neurology and some psychiatrists would see a deeper divide within their own subject between psychodynamic psychiatry and neuropsychiatry. The essential difference between the two disciplines is one of attitude, aptitude and training. Most neurologists feel the need to be well read in psychiatry without necessarily endorsing a psychiatric approach, and are grateful for having had the opportunity to learn voluntarily from psychiatrists such as R. T. C. Pratt and Sir Desmond Pond. Psychiatrists are expected to undertake some neurology as part of their training. There are few doctors in the Western world of neurology and psychiatry, though one of my mentors in the USA rejoiced in the title of professor of neurology, psychiatry and pharmacology.

In practice, the interface between psychiatry and neurology, whether we acknowledge it consciously or subconsciously, is one of immense stimulation; however, as is evident from the pages of this book, our approach is often inconsistent. Three excellent chapters, by Rosser on neurotransmitters, Trimble on psychopharmacology and Whatley *et al.* on genetic probes, emphasize shared research. Disorders of body image and circadian rhythms were beloved of neurologists but today are ably advanced by psychiatrists such as Cutting and Crisp. The hunt for a neuroanatomical basis for memory disturbances and psychoses, including the schizophrenias, forms the basis of several chapters, notably by Oxbury, Reveley and Toone (on laterality). Trimble rightly emphasizes that conversion hysteria is mainly to be found in a neurological setting and introduces the useful term AIB (abnormal illness behaviour) for conditions such as malingering, hysteria and the Münschausen syndrome where the doctor does not believe that the sick role the patient assumes is appropriate to the objective pathology displayed. Fenwick's approach to epilepsy and the dyscontrol syndrome may not be entirely new but it is always salutary to be reminded of the importance of accurate diagnosis, the recognition of the patient's social environment and the need to examine those factors which may either trigger attacks or modify their presentation. I am not totally sure to what extent a balanced presentation was planned. Benson, in looking at disorders of verbal expression in neuropsychiatry, does not explore deeply enough into language disturbances presenting in a psychiatric context. Hospitals for the mentally retarded and psychiatric units such as that for the deaf near Preston provide a happy hunting ground for neurologists seeking to learn about central language disorders. Denis Hill, on States of consciousness, examines vigilance, awareness and self, quoting the complex and philosophical book by Popper and Eccles, *The Self and Its Brain* (1977), but not that by the less contradictory Sherringtonian physiologist, Ragnar Granit, *The Purposive Brain*, published at about the same time. And Crisp, on Sleep and its disturbances, omits all mention of sleep apnoea syndromes, daytime drowsiness and narcolepsy, all of which have immense psychiatric as well as neurological implications. A counterblast, as from David Parkes' excellent monograph on sleep disturbances, is surely required.

The purpose of *The Bridge* is to stimulate thought and attitude. Each chapter is a little too short to constitute a comprehensive review and many other subjects, such as dyslexia, lying at the interface of neurology and psychiatry remain unexplored. Though not a textbook, it is nonetheless well worth reading. At a time when medical students are brainwashed by rote learning and clinical medicine must vie with administrative audit and bran tub economics, it is gratifying to read a text that stimulates what Hercule Poirot called 'those little grey cells'. If the medical profession is knocked into the ditch by political thugs, at least we can look up at the stars.

E. M. R. CRITCHLEY

Developmental Neuropathology. By Reinhard L. Friede. Second revised and expanded edition. 1989. Pp. 577. Heidelberg: Springer. Price DM 248.00

As one who has grown up with Professor Friede's monumental volume, *Developmental Neuropathology*, and through my everyday work became familiar, if not intimate, with its subtle blend of deep scholarship, sound experience and carefully considered opinion, I have waited impatiently for a new edition. In this age of multiauthored volumes where weight and cost greatly increase the likelihood of a hernia and an overdraft, the first edition of Friede's book proved a formidable exception. For the English reader it unlocked the secrets of the early German masters and with a clear-eyed approach set their work and more recent case reports into the context of modern advances in basic neuroscience. To compress the variety and complexity of the neuropathology of fetal, neonatal and infantile life into a relatively small volume was remarkable enough in 1975; it is just short of 'leger-demain' in this second edition over a decade later. The book's length has only increased by 10%, yet a wealth of new information has been added. To achieve this, some reorganization and a considerable text revision has been carried out. 'Worked over for brevity' states Friede in the preface; but there has also been a great improvement in the English style which is far more felicitous in this new edition, with only the occasional Germanicism 'on hand' to mar its clarity. Occasionally though, the quest for brevity has been overenthusiastic, justifiable reserve giving way to unwarranted dogma. For example, Brun's assertion that the dentate malformation in pontoneocerebellar hypoplasia represents the persistence of its early fetal appearance is correctly attributed in the first edition: in the second it has become the accepted wisdom despite evidence in the literature to the contrary.

Professor Friede's book is divided into 3 parts. After an extended introduction elucidating the changing morphology and reactions of the developing nervous system, the first part summarizes in a most sober fashion the myriad acquired lesions which beset the developing brain, from the encephaloclastic disorders of early gestation to hydrocephalus. This, and the second section delineating the variety and subtlety of CNS malformations, are in my opinion without parallel in the current literature for their clarity of description and balance of opinion. Current embryological advances are interpreted with great skill to illuminate the pathogenetic mechanisms underlying these anomalies. If I have a quibble it is only that the illustrations are too few They are of exceptional quality, and Springer must be congratulated for their high standard of production throughout. But even with Friede's descriptive skill, malformations are difficult for many to visualize, particularly the nonpathologist, and this subject lends itself especially well to pictorial treatment.

Many new syndromes have been smoothly integrated into the text, but I think tuberose sclerosis deserves more attention than just a passing reference in the section on cortical dysplasia. Another slight disappointment is the relatively small space given to system disorders. In the first edition they were deliberately excluded on the grounds of their similarity to more common diseases in adults. But with increasing awareness of the unique features of degenerations in early life, this claim is losing validity, and they deserve a more logically organized treatment than they are given here. It has proved impossible for Friede to ignore them, but they are scattered through the text, so that references to olivopontocerebellar atrophy occur with pontosubicular necrosis and with pontoneocerebellar hypoplasia. Striatal degeneration, here called necrosis, occurs in the acquired lesions although most are familial and some probably inborn errors, while the motor neuron degenerations are briefly referred to in the chapter on brainstem dysplasias.

The third section, on metabolic disorders, is the least successful part of the book. Here one can perhaps detect a slight lack of the personal experience so evident throughout the rest of the book. The choice of cited literature is less sure and at times capricious, but I like very much his new parcellation of diseases into organelle-related chapters, allowing the reader a logical progression through lysosomal, peroxisomal and mitochondrial disorders. This tidy organization is very appealing but I detect a slight excess of zeal in including Menkes' and Alpers' diseases in the catalogue of mitochondrial diseases.

The Alpers section is a tiny disaster The fascinating though elliptical discussion in the first edition rightly reflects the confusion present at that time. But there has been a growing literature indicating that a major part of these cases form a distinct disorder of liver and brain with characteristic patterns of clinical disease, electrophysiological and radiological abnormality, and typical pathological features. This you will not glean from the present text. Rather, Friede is excited by the possibility of a new mitochondrial disorder. He quotes my friend Joseph Egger on mitochondrial inheritance (although his paper on this topic did not refer to children with Alpers disease) and those papers which demonstrate biochemical evidence for mitochondrial disorder but in the context of only tentative morphological evidence for the diagnosis.

These are, however, minor complaints I have thoroughly enjoyed rereading Friede and I urge all serious students of neurological disease in childhood, clinician, pathologist and experimentalist alike, to obtain their copy as soon as practicable. It is essential reading.

BRIAN HARDING

Handbook of Neuropsychology. Volume 1. Edited by F. Boller, J. Grafman, G. Rizzolatti and H. Goodglass. 1988. Pp. 441 Price $181.50. Volume 2. Edited by H. Goodglass and A. R. Damasio. 1989. Pp. 436. Price $200.00. Amsterdam: Elsevier

Neuropsychology has always lacked a comprehensive handbook and even the best texts fail to cover some topics adequately or even at all. The present series, of which volumes 1 and 2 have now appeared, is therefore welcome as long as it does a good job. Does it? On the evidence of these two volumes the answer is a guarded 'Yes'. Volume 1 covers general background (7 chapters), attention (5 chapters) and the first half of language and aphasia (10 chapters). Volume 2 completes language (13 chapters) and also covers disorders of visual behaviour (9 chapters). Each of these major sections had its own editor, whose tasks presumably included such mundane things as the elimination of extensive overlap and ensuring that the major topics were covered, as well as more challenging issues such as keeping theoretical and conceptual issues to the forefront. On the whole the former is achieved whereas the latter is patchy. I found only one general and serious omission, namely that neither volume makes any serious and sustained attempt to include ideas derived from cognitive neuropsychology in the evaluation of neuropsychological disorders. For example, there is now a wealth of ideas on computational aspects of vision and memory but one would look here in vain for their relevance to neuropsychological disorders. Nor are concepts and models such as top-down vs bottom-up processing or perceptual categorization, given much more than lip service except by a few contributors.

To label the first section an 'Introduction' was perhaps a misnomer. Apart from the chapter by Benton, who provides a delightful thumbnail sketch of neuropsychology, and by Sergent, who grapples with theoretical and methodological issues without quite winning the wrestling match, the other chapters in this section seem to be there because they do not fit easily elsewhere. Thus we have contributions on the anatomy and on the assessment of cerebral dominance, classification, and bedside examination. All are informative and the busy reader may specially welcome Lezak's contribution on tests and assessment if only because it provides briefer descriptions than her much consulted book.

Section 2 deals with attention. Compared with other sections I feel that it is far too brief. It nicely covers confusional states, and what could be described as the admirable 'Italian' view of attention as exemplified by notable contributions on orienting of attention, on hemineglect, and on mechanisms and theories. But it fails to deal effectively with the challenging problem of relating clinical neglect, even speculatively, to the enormous body of experimental work on involuntary and selective attention in experimental psychology. Furthermore, it is predominantly concerned with visual neglect but without indicating that other neglects may be just as disabling for the patient or as scientifically enlightening.

The last section of Volume 1 and the first of Volume 2 represents a truly comprehensive coverage of language disorders, chiefly aphasia and dysphasia. If I had known nothing about these subjects before reading these two sections I think that they would have given me a better understanding of the history and present state of this fascinating and clinically important problem than any other account. Almost everything is covered, e.g., theories, relation to models of language, varieties of the disorder, methods of compensating, relation to reading and writing, electrophysiological correlates, selective effects in polyglots, nonverbal and other associated impairments. There are even cameos on motor control and apraxia that would justify their inclusion even if they were unrelated to aphasia. Finally, the chapter by Helen Damasio on neuroimaging is an excellent down-to-earth introduction to these increasingly common but still misunderstood techniques. It would have been just as relevant in any of the other sections.

The remainder of Volume 2 concerns disorders of visual behaviour. Perhaps more than any other section it attempts to relate clinical disorders to neurobiological work, chiefly on monkeys, by including two splendid chapters on the anatomy and physiology of cortical and subcortical visual pathways and areas It also has comprehensive treatment of well-known topics such as visual recognition (chiefly the agnosias), visuospatial disorders, visual neglect, and constructional apraxia, as well as contributions on the much more specialized topics of blindsight, mental imagery, and impaired stereo-optic depth perception All are interesting but are they any more so than conspicuous absentees such as the perception of colour or movement, or disorders of eye movements?

The *Handbook of Neuropsychology* is a major publication that is bound to be consulted for many years. It is tailor-made for students of clinical psychology, for behavioural neuroscientists who work in related areas, and for clinical neurologists Those who teach these subjects will certainly want to consult it. Unfortunately, it looks like being a runaway worst seller. At $181.50 and $200, it is hard to see who will buy these volumes except wealthy collectors and specialist libraries. And that is a pity because it deserves to be much more widely read than this will allow.

ALAN COWEY

Neurobiology of Neocortex. Edited by P. Rakic and W. Singer. 1988. Pp. 461. Chichester and New York: John Wiley. Price £50.00.

Neurobiology of Neocortex is the report of the Dahlem Workshop held in Berlin in May 1987, and the format of the book reflects the organization of the meeting. There are 4 sections, each consisting of a series of background papers that culminate in a group report by a team of 'the most internationally distinguished scientists'. The first section examines the generation of the adult neocortex during ontogenetic and phylogenetic development. Particularly noteworthy are the contributions by P. Rakic (Intrinsic and extrinsic determinants of neocortical parcellation: a radial unit model) and J. Kaas (Development of cortical sensory maps). It is a pity that recent papers on the use of retroviruses for the clonal analysis of neocortical development could not be discussed; they appeared after the book went to press, and may require the revision of some aspects of Rakic's radial unit model. Subsequent sections are concerned with the structural and biochemical organization of cortical networks, the functional organization of different cortical areas, and integrative processes in the cortex. Many of the authors provide very interesting and insightful reviews of their areas of research.

This book focuses on some of the more exciting and novel aspects of current neocortical research, and it is not intended to cover all contemporary studies of the neocortex. Indeed, the main aim of the Dahlem Workshops extends beyond simply reviewing the current status of research on a set of related topics. According to the book's detailed and rather pompous preamble, the concept of the Dahlem Workshops was created to meet the need for a multidisciplinary approach to 'define and discuss priorities and to indicate directions for future research'. This is certainly a highly commendable aim, although sadly a number of contributions, while excellent sources of information, are little more than straightforward summaries of their authors' subjects of research. In some chapters a determined effort has been made to take up the challenge posed by the organizers of the conference. Unfortunately, in some of these chapters there are a number of pitfalls for the unwary uninitiated reader. In attempting to identify major questions, elaborate theories are sometimes built on 'facts' that may really only be opinions. For example, in the Group Report of Stryker *et al.* on the 'Principles of cortical self-organization', the following statement appears: 'Corticothalamic and thalamocortical topography are both evident *in utero* in the visual cortex . . .'; the species under discussion is not mentioned and no reference is given. This statement must at best be based on slim evidence and almost certainly represents a considerable oversimplification, which is not surprising in view of the technical difficulties of in utero surgery. Similar shortcomings are to be found elsewhere. A particularly glaring omission is the lack of any references at all in the group report of Gilbert *et al.* on 'Neuronal and synaptic organization in the cortex', making a critical appraisal of its contents very difficult. Thus some of the chapters will be rather unhelpful or even misleading to the nonspecialist.

There should, however, be much interest for the specialist in the crude spontaneity evident particularly in the group reports, and the synthesis of ideas based on the multidisciplinary approach. One has the impression that the book has not only recorded the scientific content of the meeting, but has captured something of its atmosphere. Inaccuracies and idiosyncracies are to be expected in the report of a meeting of this type, and overall, the book is a considerable success. The Dahlem Workshops clearly fulfil a useful role in the promotion of scientific communication, and it is to be hoped that the series will be able to continue despite recent organizational problems (*Science*, 1989, **245**, 122)

DAVID J PRICE

Structure of the Human Brain: A Photographic Atlas. By Stephen J. DeArmond, Madeline M. Fusco and Maynard M. Dewey. Third edition. 1989 Pp. 202. New York: Oxford University Press. Price £15.00 (paperback).

This atlas consists mainly of views of series of sections through the CNS. Some of these show gross sections, useful for correlation with neuroradiological images. Others show low power views of microscopic sections, mainly through the diencephalon, brainstem and spinal cord. It also includes a series of photographs of the external features of the gross brain. The last part of the atlas illustrates cerebral angiograms and relates these to diagrams of the vasculature of the brain.

This new edition has been considerably updated, compared with the second. The series of coronal sections of the gross brain is new and complements the series of horizontal sections retained from the previous edition. A new section is included on neural chemoarchitecture. This is concerned with the distribution of histochemically defined neurons in the brainstem and diencephalon. It provides a useful summary of this rapidly expanding field. Much of the material is based on extrapolation from animal studies to the human brain. To compensate for the addition of this new material, equivalent amounts have been removed. As a result, this edition is similar in size to its predecessor

The layout of the illustrations is similar to that in the second edition. An unlabelled photograph and a detailed labelled diagram are matched on opposing pages and are generally accompanied by a small insert which clearly places the area illustrated in relation to the CNS as a whole. These correlate well. The photographs are generally of high quality and are clearly reproduced. This is especially the case with those showing the external features of the brain. However, some of those showing horizontal sections through the cerebrum fade a little in places. The quality of the material illustrated is generally very good, though a small number of the microscopic sections retained from the previous edition have slight imperfections.

This is a useful reference atlas which provides a number of coherent systematic series of sections through the CNS. The sections through the gross brain will be of considerable value for correlation with neuroradiological images. However, their potential usefulness is somewhat limited because only a few of the latter are illustrated. The microscopic sections can be studied in various degrees of depth. They will be mainly useful in fleshing out the details of pathways commonly illustrated as line diagrams in undergraduate texts. The book therefore has a broad appeal and is relatively good value for money.

JOHN P. FRAHER

Atlas of Neurosurgical Anatomy. The Pterional Perspective. By John L. Fox. 1989. Pp. 208. Heidelberg· Springer. Price DM 548.00.

No textbook of anatomy reproduces intracranial structures as seen from the neurosurgeon's eye. Hence the value of this beautifully illustrated volume, detailing the anatomical structures encountered with the pterional approach. With his extensive experience of aneurysm surgery, John Fox has compiled a series of 128 top quality colour photographs, taken through the operating microscope, guiding the reader through the variety of cisterns. For clarity, an artist's reproduction underlies each photograph, in which every structure within the field is clearly labelled.

After an introductory chapter on the history of the pterional approach, a detailed description of microsurgical instruments follows with useful advice on how to obtain most benefit from each. A brief chapter on photography through the microscope provides first-hand information for anyone wishing to develop this interest. The next chapter provides a detailed description of the technique of turning a pterional flap, and the initial intracranial approach. This precedes the principal chapters on the sylvian fissure, the carotid cistern, the chiasmatic cistern, and finally the ambient and interpeduncular cisterns. All illustrations view the approach from the right side. Aneurysms are evident in many, but tumours have been omitted since these may extensively distort the normal anatomy.

This is a superb book to supplement 'hands on' experience of the neurosurgical trainee. I can find little fault; perhaps some photographs too closely resemble their predecessors, giving a degree of repetition. Unfortunately, the quality of the book's presentation involves expense; only this should limit its sale. Anyone embarking on aneurysm surgery, or indeed on any operation involving this approach, should find this book an invaluable aid.

K. W. LINDSAY

The Cholinergic Synapse. Edited by V. P. Whittaker. *Handbook of Experimental Pharmacology,* Volume 86. 1988. Pp. 762. Berlin: Springer. Price DM 680.00.

This well-known series of volumes from Springer are, with a few noteworthy exceptions, not handbooks nor overly concerned with experimental pharmacology. Volume 86 is no different, being a collection of review articles of varying relevance and originality around the theme of acetylcholine. Conspicuously lacking is a coherent systemization of information about the cholinergic systems in a tabular form for easy reference. More surprising, in view of current medical interest, is the patchy treatment of cholinergic pharmacology, while the quantum theory, so long the flagship of cholinergic and indeed synaptic physiology in general, sinks to being perceived only as a primitive conception providing little else than nomenclature.

The lack of and need for tabular systemization is nowhere more evident than in the opening chapters in which 'model' cholinergic systems are considered. The avian ciliary ganglion which has contributed little information about the cholinergic synapse is considered at length in an individual chapter, whereas the much more important superior cervical ganglion receives only cursory treatment in the introductory 'overview'. The physiology of some peripheral cholinergic synapses are dealt with in a later section but with little concurrent attention to their anatomy and biochemistry. No clear view of the synapses of the cholinergic system emerges. One sympathizes with the editor's dilemma. Contributors to this type of volume too often disregard their duty to provide a systematic survey of the topic but see the invitation as license to grind their theoretical axes without the constraints of peer review. Who is to blame them? Their rewards will be little enough. It requires uncommon editorial persistence and hard work to insist and put together in consistent form the up-to-date anatomical, biochemical and pharmacophysiological properties of the cholinergic system. Yet it should have been done for a volume pretending to this title. If the answer is that these details can be found in a prior publication to which reference is made then we as readers are entitled to ask. why should we buy this book if the information can be found elsewhere?

Nor is the chemistry of the components of the cholinergic system better served. I could find no tabulation of the amount of acetylcholine in various tissues, parts of the brain for example. The chapter on choline-acetyltransferase contains no information on the activity in various parts of the nervous system. A chapter entitled 'Cholinesterases: *tissue* and *cellular distribution* of molecular forms' (my italics) gives no answer to the question of how much AChE is present in a given tissue. Information about pharmacological interactions with the exception of those directed towards the muscarinic receptor are either not given or buried in the text. The understanding brought about by the measurements of single channel conductances at nicotinic and muscarinic synapses receive some attenuated discussion within chapters devoted principally to other matters but, in general, the biophysics of the cholinergic system receives only cursory treatment. Presynaptic physiology is dealt with in a chapter entitled 'The neuromuscular junction' but concerned entirely with subminiature end-plate potentials that do not contribute to the evoked potential. The existence of these potentials is a substantial embarrassment for the quantum theory of synaptic transmission and in due course will force its re-evaluation. But the importance of these findings is difficult to apprehend without a reasonably detailed exposition of the quantum theory itself. Editorial authority could have been usefully exercised by arranging for the challenge to be met by an informed defence.

As a 'Handbook' this volume does not contain systematized information that cannot more readily be obtained elsewhere. How does it rate as a collection of authoritative reviews on topics of current interest? The selection of subjects and authors exposes editorial preconceptions rather more than is customary. One could hardly recommend the volume for the weight of authoritative and impartial contributions. There are disappointing lapses of scholarship; connoisseurs of double think can try to tease out the meaning of the following on p. 481 (my italics): 'A number of criteria *must* be met to consider a substance as a neurotransmitter. *None* of these criteria is adequate by itself, but it is also *unlikely* that all can be clearly established for each substance.' Finally, one might regret that there is little in this volume to indicate that the cholinergic synapse is perceived to play a role in higher, cognitive function. Much of the current interest in cholinergic systems is generated by psychologists eager to associate a physiologically defined transmitter system with a particular behaviour. A chapter or two on the uncertainties and disabilities of such labelling would have been helpful.

R. M. MARCHBANKS

ANNOUNCEMENTS

European Neurological Society

The second meeting of the European Neurological Society will be held at the Metropole Hotel, Brighton, UK, between June 30 and July 4, 1990. On June 30 and July 1 there will be six teaching courses on: Management problems in neurology I and II; Movement disorders; What you need to know about new techniques; Neuro-ophthalmology; and Neurorehabilitation. The Scientific programme (July 2–4) includes five symposia (on Retroviruses and the nervous system; Epilepsy; Basis for recovery in neurology; Behavioural neurology; and Molecular basis of neuromuscular disease), and platform and poster sessions. Further information from Concorde Services Ltd, 10 Wendell Road, London W12 9RT, UK (tel. 01 743 3106, fax 01 743 1010).

International Society for Pediatric Neurosurgery

The 18th Annual Meeting of this Society will take place at the Hotel Intercontinental, Paris, France, September 16–20, 1990. Information may be obtained from SOCFI, 14 rue Mandar, 75002 Paris. Abstracts should be submitted to Professor K. Sato, Chairman of the Programme Committee, Department of Neurological Surgery, The Juntendo University Hospital, 2-1-1. Hongo, Bunkyo-ku, Tokyo 113, Japan.

International Symposium on Pediatric Neuro-Oncology

This will be held at the Penn Tower Hotel, Philadelphia, Pennsylvania, USA, on May 24–26, 1990, sponsored by the Children's Hospital of Philadelphia, University of Pennsylvania, and the Children's Hospital and Medical Center, University of Washington, Seattle, USA.

Residential Course/Workshop on Blood Vessel Imaging using Ultrasound Techniques

This will be held at the Dolphin Hotel, Southampton, UK, on May 16–18, 1990. Details and registration forms may be obtained from Mr K. N Humphries, Blood Vessel Imaging Course, 10 Swale Drive, Chandlers Ford, Hampshire SO5 3QY, UK.

Ninth Biennial Conference of the Pan African Association for Neurological Sciences

This will take place at the Holiday Inn, Harare, Zimbabwe, on May 14–17, 1990. Further information may be obtained from Professor L. F. Levy, 5 Montagu Avenue, Harare, Zimbabwe (tel. 792020).

First International Congress on Brain Electromagnetic Topography

'Topography 90 Osaka' will be held at the Royal Hotel, Osaka, Japan, on September 12–14, 1990 Further information may be obtained from the Congress Office, Department of Neurosurgery, University of Occupational and Environmental Health, 1-1 Iseigoaka, Yahatanishi-ku, Kitakyushu, Fukuoko 807, Japan.

BIBLIOGRAPHIC REFERENCES should be limited to essential literature. They should be listed at the end of the paper in alphabetical order and not numbered. For a citation from a *journal*, the author's name in capital letters and the year of publication in brackets are followed by the title of the paper, the name of the journal *in full* and underlined for italicization, the volume number and the first and last pages of the journal. For multiple publications by the same author, those by the author alone are listed first and any with two or more co-authors listed subsequently in chronological order. The names of all authors must be given up to a maximum of six. If there is more than one paper for a given year, these should be listed *a*, *b*, *c*, etc., after the year For *books*, the author's name and year of publication in brackets are followed by the title of the book, underlined for italicization, place of publication, publisher's name and first and last page numbers. For *chapters in books*, the author's name and year of publication in brackets is followed by the title of the chapter, the title of the book (underlined), preceded by 'In:', the name(s) of the editor(s), the place of publication, the name of the publishers and the first and last pages of the chapter *In the text*, numbered references are not used. The author's name and year of publication are given in brackets If there are three or more authors, the name of the first is followed by *et al*. The punctuation, both in the Reference list and in the text, should follow the style of the journal (*see* current issue). Papers in which the reference citations do not follow this format may be returned for retyping. References to papers 'in preparation' or 'submitted' are not acceptable, if 'in press' the name of the journal or book must be given Reference citations should not include 'personal communications' or other inaccessible information; information derived from personal communications or from unpublished work by the authors should be referred to in the text.

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ABBREVIATIONS FOR SCIENTIFIC UNITS should conform to the Système Internationale (SI units). The statistical guidelines advocated by the International Committee of Medical Journal Editors (*Ann. Intern. Med.* **108**, 266-273, 1988) should be followed.

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TRAVEL GRANTS

The Guarantors of Brain are prepared to consider applications for part or all of the costs incurred by clinical and nonclinical neuroscientists based in the United Kingdom wishing to visit laboratories or clinical departments at home or abroad. Two schemes exist.

1 Individuals aged under 40 years may apply for the costs of short visits. Applications will be considered for attendance at scientific meetings which relate directly to the applicant's research interests. Only exceptionally will applications to attend larger international conferences be considered. Awards of up to £1000 will be made throughout the year. The Guarantors encourage applicants to meet part of their costs from other sources. Applications should be submitted at least two months before the planned date of departure.

2 Individuals of any age may apply for a limited number of awards which will enable a neuroscientist to spend several weeks visiting a clinical department or laboratory in order to learn new techniques which will subsequently be used to develop or supplement existing research. Up to £5000 will be available to cover travel, subsistence and recurrent expenses incurred in the host department. Applications will be considered during May and November of each year.

The following documents are required for awards under each scheme:

1. an abbreviated curriculum vitae with a list of publications;
2. an account of the purpose of the visit;
3. the names of two referees;
4. a letter of invitation from the host department;
5. a statement of the costs with details of related applications;
6. a list of previous awards given by the Guarantors of Brain.

Four copies should be sent to Professor Alastair Compston, Honorary Secretary and Treasurer to the Guarantors of Brain, Department of Neurology, Addenbrooke's Hospital, Cambridge, CB2 2QQ (Tel. 0223 217091).

All successful applicants are expected to submit a short report of the visit on their return.